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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/17 — A2

(11) International Publication Number: WO 97/33909

(43) International Publication Date: 18 September 1997 (18.09.97)

(21) International Application Number: PCT/US97/04192

(22) International Filing Date: 14 March 1997 (14.03.97)

(30) Priority Data:

08/616,745	15 March 1996 (15.03.96)	US
08/633,840	11 April 1996 (11.04.96)	US

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(81) Designated States: AL, AM, AT, AU, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

(57) Abstract

Compounds and methods for treating and diagnosing prostate cancer are provided. The inventive compounds include polypeptides containing at least a portion of a prostate protein. Vaccines and pharmaceutical compositions for immunotherapy of prostate cancer comprising such polypeptides or DNA molecules encoding such polypeptides are also provided. The inventive polypeptides may also be used to generate antibodies useful for the diagnosis and monitoring of prostate cancer. Nucleic acid sequences for preparing probes, primers, and polypeptides are also provided.

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COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

TECHNICAL FIELD

The present invention relates generally to the treatment, diagnosis and monitoring of prostate cancer. The invention is more particularly related to polypeptides comprising at least a portion of a prostate protein. Such polypeptides may be used in vaccines and pharmaceutical compositions for treatment of prostate cancer. The polypeptides may also be used for the production of compounds, such as antibodies, useful for diagnosing and monitoring the progression of prostate cancer, and possibly other tumor types, in a patient.

BACKGROUND OF THE INVENTION

Prostate cancer is the most common form of cancer among males, with an estimated incidence of 30% in men over the age of 50. Overwhelming clinical evidence shows that human prostate cancer has the propensity to metastasize to bone, and the disease appears to progress inevitably from androgen dependent to androgen refractory status, leading to increased patient mortality. This prevalent disease is currently the second leading cause of cancer death among men in the U.S.

In spite of considerable research into therapies for the disease, prostate cancer remains difficult to treat. Commonly, treatment is based on surgery and/or radiation therapy, but these methods are ineffective in a significant percentage of cases. Three prostate specific proteins - prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) - have limited diagnostic and therapeutic potential. PSA levels do not always correlate well with the presence of prostate cancer, being positive in a percentage of non-prostate cancer cases, including benign prostatic hyperplasia (BPH). Furthermore, PSA measurements correlate with prostate volume, and do not indicate the level of metastasis.

Accordingly, there remains a need in the art for improved vaccines and diagnostic methods for prostrate cancer.

SUMMARY OF THE INVENTION

The present invention provides compounds and methods for immunotherapy and diagnosis of prostate cancer. In one aspect, polypeptides are provided comprising at least an immunogenic portion of a prostate protein having a partial sequence as provided in SEQ ID Nos. 2 and 4-8, or a variant of such a protein that differs only in conservative substitutions and/or modifications.

In related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided. In preferred embodiments, the host cells are selected from the group consisting of *E. coli*, yeast and mammalian cells.

The present invention also provides pharmaceutical compositions comprising one or more of the polypeptides of SEQ ID Nos. 1-8, 20, 21, 25-31 or 44-57, or nucleic acids of SEQ ID Nos. 9-19, 22-24 or 32-43, and a physiologically acceptable carrier. The invention also provides vaccines comprising one or more of such polypeptides or nucleic acids in combination with a non-specific immune response enhancer.

In yet another aspect, methods are provided for inhibiting the development of prostate cancer in a patient, comprising administering an effective amount of one or more of the polypeptides of SEQ ID Nos. 1-8, 20, 21, 25-31 or 44-57, or nucleic acids of SEQ ID Nos. 9-19, 22-24 or 32-43 to a patient in need thereof.

In further aspects, methods are provided for detecting prostate cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to a polypeptide of SEQ ID Nos. 1-8, 20, 21, 25-31 or 44-57; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent.

In related aspects, methods are provided for monitoring the progression of prostate cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to a polypeptide of SEQ

ID Nos. 1-8, 20, 21, 25-31 or 44-57; (b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent; (c) repeating steps (a) and (b); and comparing the amounts of polypeptide detected in steps (b) and (c).

Within related aspects, the present invention provides antibodies, preferably monoclonal antibodies, that bind to the polypeptides described above, as well as diagnostic kits comprising such antibodies, and methods of using such antibodies to inhibit the development of prostate cancer.

The present invention also provides methods for detecting prostate cancer comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and (c) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primer. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

In a further aspect, the present invention provides a method for detecting prostate cancer in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and (c) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a Western blot analysis of sera obtained form rats immunized with rate prostate extract.

Fig. 2 illustrates a non-reduced SDS PAGE of the rat immunizing preparation of Fig. 1.

Fig. 3 illustrates the binding of a putative human homologue of rat steroid binding protein to progesterone and to estramustine.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the immunotherapy, diagnosis and monitoring of prostate cancer. The inventive compositions are generally polypeptides that comprise at least a portion of a human prostate protein, the protein demonstrating immunoreactivity with human prostate sera. Also included within the present invention are molecules (such as an antibody or fragment thereof) that bind to the inventive polypeptides. Such molecules are referred to herein as "binding agents."

In particular, the subject invention discloses polypeptides comprising at least a portion of a human prostate protein provided in SEQ ID Nos. 2 and 4-8, or a variant of such a protein that differs only in conservative substitutions and/or modifications. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising a portion of one of the above prostate proteins may consist entirely of the portion, or the portion may be present within a larger polypeptide that contains additional sequences. The additional sequences may be derived from the native protein or may be heterologous, and such sequences may be immunoreactive and/or antigenic.

As used herein, an "immunogenic portion" of a human prostate protein is a portion that reacts either with sera derived from an individual inflicted with autoimmune prostatitis or with sera derived from a rat model of autoimmune prostatitis. In other words, an immunogenic portion is capable of eliciting an immune response and

as such binds to antibodies present within prostatitis sera. Autoimmune prostatitis may occur, for example, following treatment of bladder cancer by administration of Bacillus Calmette-Guerin (BCG), an avirulent strain of *Mycobacterium bovis*. In the rat model of autoimmune prostatitis, rats are immunized with a detergent extract of rat prostate. Sera from either of these sources may be used to react with the human prostate derived polypeptides described herein. Antibody binding assays may generally be performed using any of a variety of means known to those of ordinary skill in the art, as described, for example, in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988. For example, a polypeptide may be immobilized on a solid support (as described below) and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

A "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the immunotherapeutic, antigenic and/or diagnostic properties of the polypeptide or molecules that bind to the polypeptide, are retained. For prostate proteins with immunoreactive properties, variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the immunoreactivity of the modified polypeptide. For prostate proteins useful for the generation of diagnostic binding agents, a variant may be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of prostate cancer. Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu,

asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides having one of the sequences provided in SEQ ID Nos. 1 to 8, 20, 21 and 25-31 may be isolated from a suitable human prostate adenocarcinoma cell line, such as LnCap.fgc (ATCC No. 1740-CRL). LnCap.fgc is a prostate adenocarcinoma cell line that is a particularly good representation of human prostate cancer. Like the human cancer, LnCap.fgc cells form progressively growing tumors as xenografts in SCID mice, respond to testosterone, secrete PSA and respond to the presence of bone marrow components (*e.g.*, transferrin). In particular, the polypeptides may be isolated by expression screening of a LnCap.fgc cDNA library with human prostatitis sera using techniques described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein), and as described in detail below. The polypeptides of SEQ ID No. 48 and 49 may be isolated from the LnCap/fgc cell line by screening with sera from the rat model of autoimmune prostatitis discussed above. The polypeptides of SEQ ID Nos. 50-56 may be isolated from the LnCap/fgc cell line by screening with human prostatitis sera as described in detail in Example 4. The polypeptides of SEQ ID No. 44-47 may be isolated from human seminal fluid as described in detail in Example 2. Once a DNA sequence encoding a polypeptide is obtained, any of the above modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis.

The polypeptides disclosed herein may also be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc., (Foster City, CA), and may be operated according to the manufacturer's instructions.

Alternatively, any of the above polypeptides may be produced recombinantly by inserting a DNA sequence that encodes the polypeptide into an expression vector and expressing the protein in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as CHO cells. The DNA sequences expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in substantially pure form (*i.e.*, the polypeptides are homogenous as determined by amino acid composition and primary sequence analysis). Preferably, the polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in more detail below, the substantially pure polypeptides are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

Polypeptides of the present invention that comprise an immunogenic portion of a prostate protein may generally be used for immunotherapy of prostate cancer, wherein the polypeptide stimulates the patient's own immune response to prostate tumor cells. In further aspects, the present invention provides methods for using one or more of the immunoreactive polypeptides of SEQ ID Nos. 1 to 8, 20, 21, 25-31 and 44-57 (or DNA encoding such polypeptides) for immunotherapy of prostate cancer in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease. Accordingly, the above immunoreactive polypeptides may be used to treat prostate cancer or to inhibit the development of prostate cancer. The polypeptides may be administered either prior to or following surgical removal of primary tumors and/or treatment by administration of radiotherapy and conventional chemotherapuetic drugs.

In these aspects, the polypeptide is generally present within a pharmaceutical composition and/or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. The vaccines may comprise one or more of such polypeptides and a non-specific immune response enhancer, such as an adjuvant, biodegradable microsphere (*e.g.*, polylactic galactide) or a liposome (into which the polypeptide is incorporated). Pharmaceutical compositions and vaccines may also contain other epitopes of prostate cell antigens, either incorporated into a combination polypeptide (*i.e.*, a single polypeptide that contains multiple epitopes) or present within a separate polypeptide.

Alternatively, a pharmaceutical composition or vaccine may contain DNA encoding one or more of the above polypeptides, such that the polypeptide is generated *in situ*. In such pharmaceutical compositions and vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter). Bacterial delivery

systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an epitope of a prostate cell antigen on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *PNAS 86*:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner et al., *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld et al., *Science 252*:431-434, 1991; Kolls et al., *PNAS 91*:215-219, 1994; Kass-Eisler et al., *PNAS 90*:11498-11502, 1993; Guzman et al., *Circulation 88*:2838-2848, 1993; and Guzman et al., *Cir. Res. 73*:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in published PCT application WO 90/11092, and Ulmer et al., *Science 259*:1745-1749, 1993, reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Routes and frequency of administration, as well as dosage, will vary from individual to individual and may parallel those currently being used in immunotherapy of other diseases. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 10 doses may be administered over a 3-24 week period. Preferably, 4 doses are administered, at an interval of 3 months, and booster administrations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that is effective to raise an immune response (cellular and/or humoral) against prostate tumor cells in a treated patient. A suitable immune response is at least 10-50% above the basal (*i.e.*, untreated) level. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in

a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.01 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax and/or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and/or magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic glycolide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of non-specific immune response enhancers may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*. Such adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).

Polypeptides disclosed herein may also be employed in *ex vivo* treatment of prostate cancer. For example, cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as CellPro Incorporated's (Bothell, WA) CEPRATE™ system (see U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of tumor antigen-

specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

Polypeptides of the present invention may also, or alternatively, be used to generate binding agents, such as antibodies or fragments thereof, that are capable of detecting metastatic human prostate tumors.

Binding agents of the present invention may generally be prepared using methods known to those of ordinary skill in the art, including the representative procedures described herein. Binding agents are capable of differentiating between patients with and without prostate cancer, using the representative assays described herein. In other words, antibodies or other binding agents raised against a prostate protein, or a suitable portion thereof, will generate a signal indicating the presence of primary or metastatic prostate cancer in at least about 20% of patients afflicted with the disease, and will generate a signal indicating the absence of the disease in at least about 90% of individuals without primary or metastatic prostate cancer. Suitable portions of such prostate proteins are portions that are able to generate a binding agent that indicates the presence of primary or metastatic prostate cancer in substantially all (*i.e.*, at least about 80%, and preferably at least about 90%) of the patients for which prostate cancer would be indicated using the full length protein, and that indicate the absence of prostate cancer in substantially all of those samples that would be negative when tested with full length protein. The representative assays described below, such as the two-antibody sandwich assay, may generally be employed for evaluating the ability of a binding agent to detect metastatic human prostate tumors.

The ability of a polypeptide prepared as described herein to generate antibodies capable of detecting primary or metastatic human prostate tumors may generally be evaluated by raising one or more antibodies against the polypeptide (using, for example, a representative method described herein) and determining the ability of such antibodies to detect such tumors in patients. This determination may be made by assaying biological samples from patients with and without primary or metastatic prostate cancer for the presence of a polypeptide that binds to the generated antibodies. Such test assays may be performed, for example, using a representative procedure

described below. Polypeptides that generate antibodies capable of detecting at least 20% of primary or metastatic prostate tumors by such procedures are considered to be able to generate antibodies capable of detecting primary or metastatic human prostate tumors. Polypeptide specific antibodies may be used alone or in combination to improve sensitivity.

Polypeptides capable of detecting primary or metastatic human prostate tumors may be used as markers for diagnosing prostate cancer or for monitoring disease progression in patients. In one embodiment, prostate cancer in a patient may be diagnosed by evaluating a biological sample obtained from the patient for the level of one or more of the above polypeptides, relative to a predetermined cut-off value. As used herein, suitable "biological samples" include blood, sera, urine and/or prostate secretions.

The level of one or more of the above polypeptides may be evaluated using any binding agent specific for the polypeptide(s). A "binding agent," in the context of this invention, is any agent (such as a compound or a cell) that binds to a polypeptide as described above. As used herein, "binding" refers to a noncovalent association between two separate molecules (each of which may be free (*i.e.*, in solution) or present on the surface of a cell or a solid support), such that a "complex" is formed. Such a complex may be free or immobilized (either covalently or noncovalently) on a support material. The ability to bind may generally be evaluated by determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind" in the context of the present invention when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known to those of ordinary skill in the art.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome with or without a peptide component, an RNA molecule or a peptide. In a preferred embodiment, the binding partner is an antibody, or a fragment thereof. Such antibodies may be polyclonal, or monoclonal. In

addition, the antibodies may be single chain, chimeric, CDR-grafted or humanized. Antibodies may be prepared by the methods described herein and by other methods well known to those of skill in the art.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding partner to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of binding partner immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a second binding partner that contains a reporter group. Suitable second binding partners include antibodies that bind to the binding partner/polypeptide complex. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding partner after incubation of the binding partner with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding partner is indicative of the reactivity of the sample with the immobilized binding partner.

The solid support may be any material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent,

in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μg, and preferably about 100 ng to about 1 μg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see*, *e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a second antibody (containing a reporter group) capable of binding to a different site on the polypeptide is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact

time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with prostate cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of antibody to reporter group may be achieved using standard methods known to those of ordinary skill in the art.

The second antibody is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound second antibody is then removed and bound second antibody is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of prostate cancer, the signal detected from the reporter group that remains bound to the solid support is generally

compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without prostate cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for prostate cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for prostate cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antibody is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized antibody as the sample passes through the membrane. A second, labeled antibody then binds to the antibody-polypeptide complex as a solution containing the second antibody flows through the membrane. The detection of bound second antibody may then be performed as described above. In the strip test format, one end of the membrane to which antibody is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second antibody and to the area of immobilized antibody. Concentration of second antibody at the area of immobilized antibody indicates the presence of prostate cancer. Typically, the concentration of second antibody at that site generates a pattern, such as a line, that can

be read visually. The absence of such a pattern indicates a negative result. In general, the amount of antibody immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the antigens or antibodies of the present invention. The above descriptions are intended to be exemplary only.

In another embodiment, the above polypeptides may be used as markers for the progression of prostate cancer. In this embodiment, assays as described above for the diagnosis of prostate cancer may be performed over time, and the change in the level of reactive polypeptide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, prostate cancer is progressing in those patients in whom the level of polypeptide detected by the binding agent increases over time. In contrast, prostate cancer is not progressing when the level of reactive polypeptide either remains constant or decreases with time.

Antibodies for use in the above methods may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating

one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Monoclonal antibodies of the present invention may also be used as therapeutic reagents, to diminish or eliminate prostate tumors. The antibodies may be

used on their own (for instance, to inhibit metastases) or coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of

different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the

precise does of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify prostate tumor-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80% identity, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect prostate and/or prostate tumor sequences in biological samples, preferably blood, semen or prostate and/or prostate tumor tissue.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

A. Isolation of Polypeptides from LnCap.fgc using human prostatitis sera

Representative polypeptides of the present invention were isolated by screening a human prostate cancer cell line with human prostatitis sera as follows. A human prostate adenocarcinoma cDNA expression library was constructed by reverse transcriptase synthesis from mRNA purified from the human prostate adenocarcinoma cell line LnCap.fgc (ATCC No. 1740-CRL), followed by insertion of the resulting cDNA clones in Lambda ZAP II (Stratagene, La Jolla, CA).

Human prostatitis serum was obtained from a patient diagnosed with autoimmune prostatitis following treatment of bladder carcinoma by administration of BCG. This serum was used to screen the LnCap cDNA library as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. Specifically, LB plates were overlaid with approximately 10^4 pfu of the LnCap cDNA library and incubated at 42°C for 4 hours prior to obtaining a first plaque lift on isopropylthio-beta-galactoside (IPTG) impregnated nitrocellulose filters. The plates were then incubated for an additional 5 hours at 42°C and a second plaque lift was prepared by incubation overnight at 37°C. The filters were washed three times with PBS-T, blocked for 1 hours with PBS (containing 1% Tween 20™) and again washed three times with PBS-T, prior to incubation with human prostatitis sera at a dilution of 1:200 with agitation overnight. The filters were then washed three times with PBS-T and incubated with ^{125}I-labeled Protein A (1 µl/15 ml PBS-T) for 1 hour with agitation. Filters were exposed to film for variable times, ranging from 16 hours to 7 days. Plaques giving signals on duplicate lifts were re-plated on LB plates. Resulting plaques were lifted with duplicate filters and these filters were treated as above. The filters were incubated with human prostatitis sera (1:200 dilution) at 4°C with agitation overnight. Positive plaques were

visualized with ^{125}I-Protein A as described above with the filters being exposed to film for variable times, ranging from 16 hours to 11 days. *In vivo* excision of positive human prostatitis antigen cDNA clones was performed according to the manufacturer's protocol.

B. Characterization of Polypeptides

DNA sequence for positive clones was obtained using forward and reverse primers on an Applied Biosystems Inc. Automated Sequence Model 373A (Foster City, CA). The cDNA sequences encoding the isolated polypeptides, hereinafter referred to as HPA8, HPA13, HPA15 - HPA17, HPA20, HPA25, HPA28, HPA29, HPA32 - HPA38 and HPA41 are presented in SEQ ID Nos. 32 and 33, 34 and 35, 36, 9 and 10, 11, 12, 13 and 14, 15, 37 and 38, 16, 39, 22 and 23, 17 and 18, 19, 24, 40 and 41, 42 and 43, respectively. The 3' sequences of HPA16 and HPA20 are identical. HPA13, HPA16, HPA20, HPA29 and HPA33 are believed to be overlapping clones with novel 5' end points. Two of the positive clones were determined to be identical to HPA15. Also, HPA15, HPA34 and HPA37 were found to be overlapping clones. The expected N-terminal amino acid sequences of the isolated polypeptides HPA16, HPA17, HPA20, HPA25, HPA28, HPA32, HPA35, HPA36, HPA34, HPA37, HPA8, HPA13, HPA15, HPA29, HPA33, HPA38 and HPA41, based on the determined cDNA sequences in frame with the N-terminal portion of β-galactosidase (lacZ) are presented in SEQ ID Nos. 1-8, 20, 21 and 25-31, respectively.

The determined cDNA and expected amino acid sequences for the isolated polypeptides were compared to known sequences in the gene bank using the EMBL and GenBank (Release 91) databases, and also the DNA STAR system. The DNA STAR system is a combination of the Swiss, PIR databases along with translated protein sequences (Release 91). No significant homologies to HPA17, HPA25, HPA28, HPA32, HPA35 and HPA36 were found.

The determined cDNA sequence for HPA8 was found to have approximately 100% identity with the human proto-oncogene BMI-1 (Alkema, M.J. et al., *Hum. Mol. Gen.* 2:1597-1603, 1993). Search of the DNA database with 5' and 3'

cDNA sequence encoding HPA13 revealed 100% identity with a known cDNA sequence from a human immature myeloid cell line (GenBank Acc. No. D63880). Search of the protein database with the deduced amino acid sequence for HPA13 revealed 100% identity with the open reading frame encoded by the same human cDNA sequence. Search of the protein database with the expected amino acid sequence for HPA15, revealed high homology (60% identity) with a *Saccharomyces cerevisiae* predicted open reading frame (Swiss/PIR Acc. No. S46677), and 100% identity with a human protein from pituitary gland modulating intestinal fluid secretion (Lonnroth, I.., *J. Biol. Chem. 35*:20615-20620, 1995). The deduced amino acid sequence for HPA38 was found to have 100% identity with human heat shock factor protein 2 (Schuetz, T. J. et al., *Proc. Natl. Acad. Sci. USA 88*:6911-6915, 1991). Search of the DNA database with the 5' DNA sequence for HPA41 and search of the protein database with the deduced amino acid sequence revealed 100% identity with a human LIM protein (Rearden, A., *Biochem. Biophys. Res. Commun. 201*:1124-1131, 1994). To the best of the inventors' knowledge, except for LIM protein, none of the inventive polypeptides have been previously shown to be present in human prostate.

Positive phagemid viral particles were used to infect *E. coli* XL-1 Blue MRF', as described in Sambrook et al., *supra*. Induction of recombinant protein was accomplished by the addition of IPTG. Induced and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human prostatitis sera (1:200 dilution) and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd portion of lacZ. Sera incubations were performed for 2 hours at room temperature. Bound antibody was detected by addition of ^{125}I-labeled Protein A and subsequent exposure to film for variable times ranging from 16 hours to 11 days. The results of the immunoblots are summarized in Table I, wherein (+) indicates a positive reaction and (-) indicates no reaction.

TABLE I

Antigen	Human Prostatitis Sera	Anti-lacZ Sera	Protein Mass/Kd
HPA8	(-)	(-)	
HPA13	(+)	(+)	
HPA15	(+)	(+)	50
HPA16	(+)	(+)	40
HPA17	(+)	(-)	40
HPA20	(+)	(+)	38
HPA25	(-)	(+)	32
HPA28	(-)	(-)	
HPA29	(+)	(+)	
HPA32	(-)	(-)	
HPA33	(+)	(+)	
HPA34	not tested	(+)	50
HPA35	(-)	(-)	
HPA36	(-)	(-)	
HPA37	not tested	(+)	50
HPA38	(-)	(-)	
HPA41	not tested	(+)	

Positive reaction of the recombinant human prostatitis antigens with both the human prostatitis sera and anti-lacZ sera indicate that reactivity of the human prostatitis sera is directed towards the fusion protein. Cloned antigens showing reactivity to the human prostatitis sera but not to anti-lacZ sera indicate that the reactive protein is likely initiating within the clone. Antigens reactive with the anti-lacZ sera but not with the human prostatitis sera may be the result of the human prostatitis sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the immunoblot is not sufficient. Antigens not

reactive with either sera are not being expressed in *E. coli*, and reactive epitopes may be within the fusion protein or within an internal open reading frame. Due to the instability of recombinant antigens from HPA13, HPA29 and HPA33, it was not possible to determine the size of the recombinant antigens.

The expression of representative human prostatitis antigens was investigated by RT-PCR in four different human cell lines (including two metastatic prostate tumor lines LNCaP and DU145), normal prostate, breast, colon, kidney, stomach, lung and skeletal muscle tissue, nine different prostate tumor samples and three different breast tumor samples. The results of these studies are shown in Table II.

Table II

Analysis of HPA clone mRNA expression by RT-PCR in human cell lines, normal tissues and tumors

Clone	LNCaP	DU145	MCF-12A	HBL-100	Prostate	Breast	Colon	Kidney	Stomach	Lung	Skel. Muscle
hpa-17	+	++	+	+	+	-	±	-	-	+	+
hpa-20	+++	++++	NT	NT	±	NT	NT	-	NT	+	NT
hpa-28	+	+++	+	+	+	-	±	+	-	+	±

	Prostate Tumors (n=9)									Breast Tumors (n=3)		
Clone	Tumor 1	Tumor 2	Tumor 3	Tumor 4	Tumor 5	Tumor 6	Tumor 7	Tumor 8	Tumor 9	Tumor 1	Tumor 2	Tumor
hpa-17	+	+	+	-	+	+	±	-	-	+	++	
hpa-20	+	+	NT	NT	NT	NT	NT	NT	NT	+	+	++
hpa-28	+	+	±	-	+	+	++	±	-	++	+++	+

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mRNA expression of representative antigens in LNCaP and normal prostate, kidney, liver, stomach, lung and pancreas was also investigated by RNase protection. The results of these studies are provided in Table III.

Table III

Analysis of HPA clone mRNA expression by RNase protection in LNCaP and normal human tissues

Clone	LNCaP	Prostate	Kidney	Liver	Stomach	Lung	Pancreas
hpa-15	+	-	++	++	+	-	++
hpa-20	+++++	+	+	+	+	NT	NT
hpa-25	+	+	+	+	++	++	NT
hpa-32	NT	++	+	+	NT	++	NT
hpa-35	+++	+++	NT	+	+	+++	+
hpa-36	+	+	NT	NT	+	+	+

Example 2

A. Isolation and Characterization of Rat Steroid Binding Protein

Immune sera was obtained from rats immunized with rat prostate extract to generate antibodies to self prostate antigens. Specifically, rats were prebled to obtain control sera prior to being immunized with a detergent extract of rat prostate (in PBS containing 0.1% Triton) in Freunds complete adjuvant. A boost of incomplete Freunds adjuvant was given 3 weeks after the initial immunization and sera was harvested at 6 weeks.

The sera thus obtained was subjected to ECL Western blot analysis (Amersham International, Arlington Heights, Ill) using the manufacturer's protocol and a rat prostate protein was identified, as shown in Fig. 1. After reduction, SDS-PAGE revealed a broad silver staining band migrating at 7 kD. Without reduction, a strong band was seen at 24 kD (Fig. 2). This protein was purified by ion exchange

chromatography and subjected to gel electrophoresis under reduced conditions. Three bands were seen, indicating the presence of three chains within the protein: a 6-8 kD chain (C1), a 8-10 kD chain (C2) and a 10-12 kD chain (C3). The protein was further purified by reverse phase HPLC on a Delta™ C18 300 A° 5 μm column, column size 3.9 x 300 mm (Waters-Millipore, Milford, MA). The sample containing 100 μg of protein was dissolved in 0.1% trifluoroacetic acid (TFA), pH 1.9 and polypeptides were eluted with a linear gradient of acetonitrile (0-60%) in 0.1% TFA pH 1.9 at a flow rate of 0.5 mL/min for 1 hour. The eluent was monitored at 214 nm. Two peaks were obtained, a C1-C3 dimer and a C2-C3 dimer. The amino terminus of the C2 chain was found to be blocked. The C1 and C3 chains were sequenced on a Perkin Elmer/Applied Biosystems Inc. Procise Model 494 protein sequencer and found to have the following amino terminal sequences (Seq. ID Nos. 44 and 45, respectively).

(a) Ser-Gln-Ile-Cys-Glu-Leu-Val-Ala-His-Glu-Thr-Ile-Ser-Phe-Leu; and

(b) Xaa-Xaa-Xaa-Xaa-Xaa-Ser-Ile-Leu-asp-Glu-Val-Ile-Arg-Gly-Thr,

wherein Xaa may be any amino acid.

These sequences were compared to known sequences in the gene bank using the databases discussed in Example 1 and were found to be identical to rat steroid binding protein, also known as estramustine-binding protein (EMBP) (Forsgren, B. et al., *Prog. Clin. Biol. Res. 75A*:391-407, 1981; Forsgren, B. et al., *Proc. Natl. Acad. Sci. USA 76*:3149-53, 1979). This protein is a major secreted protein in rat seminal fluid and has been shown to bind steroid, cholesterol and proline rich proteins. EMBP has been shown to bind estramustine and estromustine, the active metabolites of estramustine phosphate. Estramustine phosphate has been found to be clinically useful in treating advanced prostate cancer in patients who do not respond to standard hormone ablation therapy (see, for example, Van Poppel, H. et al., *Prog. Clin. Biol. Res. 370*:323-41, 1991).

B. Isolation of putative human homologue to rat steroid binding protein

Purified rat steroid binding protein was obtained from freshly excised rat prostate and used to subcutaneously immunize a New Zealand white virgin female

rabbit (150 μg purified rat steroid binding protein in 1 ml of PBS and 1 ml of incomplete Freund's adjuvant containing 100 μg of muramyl dipeptide (adjuvant peptide, Calbiochem, La Jolla, CA). Six weeks later the rabbit was boosted subcutaneously with the same protein dose in incomplete Freund's adjuvant. Finally, the rabbit was boosted intravenously two weeks later with 100 μg protein in PBS and the sera harvested two weeks after the final immunization.

The resulting rabbit antisera was used to screen the LnCap.fgc cell line without success. The rabbit antisera was subsequently used to screen human seminal fluid anion exchange chromatography pools using the protocol detailed below in Example 3. This analysis indicated an approximately 18-22 kD cross-reactive protein. The seminal fluid fraction of interest (Fraction 1) was separated into individual components by SDS-PAGE under non-reducing conditions, blotted onto a PVDF membrane, excised and digested with CNBr in 70% formic acid. The resulting CNBr fragments were resolved on a tricine gel system, again electroblotted to PVDF and excised. The sequence for one peptide was determined as follows:

Val-Val-Lys-Thr-Tyr-Leu-Ile-Ser-Ser-Ile-Pro-Leu-Gln-Gly-Ala-Phe-Asn-Tyr-Lys-Tyr-Thr-Ala (SEQ. ID No. 46).

This sequence was compared to known sequences in the gene bank using the databases identified above and was unexpectedly found to be identical to gross cystic disease fluid protein, a protein whose expression was previously found to correlate with the presence of metastatic breast cancer (Murphy, L.C. et al., *J. Biol. Chem. 262*:15236-15241, 1987). To the best of the inventors' knowledge, this protein has not been previously identified in male tissues.

The ability of Fraction 1 as described above, to bind to steroid was investigated as follows. Purified rat steroid binding protein (RSBP) and fraction 1 were subjected to SDS-PAGE and transferred onto nitrocellulose filters. Specifically, 1.5 μg of RSBP/gel lane and 4 μg of fraction 1/gel lane were electrophoresed in parallel on a 4-20% gradient Laemmli gel (BioRad), then electrophoretically transferred to nitrocellulose. After protein transfer, the nitrocellulose was blocked for 1 hour at room temperature in 1% Tween 20 in PBS, rinsed three times for 10 min each in 10 ml

0.1% Tween 20 in PBS plus 0.5 M NaCl, then probed with either 1) 0.87 μM progesterone conjugated to horseradish peroxidase (HRP, Sigma) diluted in the rinse buffer; 2) 0.87 μM progesterone HRP with 200 μM estramustine; or 3) 0.87 μM progesterone HRP plus 400 μM unlabelled progesterone and 200 μM estramustine. Each reaction mixture was incubated for 1 hour at room temperature and washed three times for 10 min each with 0.1% Tween 20 , PBS, and 0.5 M NaCl. The blots were then developed (ECL system, Amersham) to reveal progesterone HRP binding proteins that are also capable of binding estramustine.

With both rat steroid binding protein and Fraction 1, three bands were obtained that bound HRP-progesterone and that were competed out with unlabelled progesterone and estramustine (Fig. 3). These results indicate that the three bands isolated from human seminal fluid as described above bind hormone and correspond in number of polypeptides to the chains C1, C2 and C3 of rat steroid binding protein, although slightly bigger in size, either due to primary sequence or secondary post-translational modifications.

This putative homologue of rat steroid binding protein was also identified in a subsequent screen of human seminal fluid using the rabbit antisera detailed above. Specifically a hydrophobic 22kD/65kD aggregate protein was obtained which, following CNBr digestion of the 22kD band, provided a peptide having the following sequence:

Val-Val-Lys-Thr-Tyr-Leu-Ile-Ser-Ser-Ile-Pro-Leu-Gln-Ala-Phe-Asn-Tyr-Lys-Tyr-Thr-Ala (SEQ. ID No. 47).

This peptide was found to correspond to residues 67 through 87 of gross cystic disease fluid protein and was identified again utilizing human autoimmune prostatitis sera as discussed below in Example 4.

Example 3

Isolation and Characterization of Polypeptides Isolated from LnCaP.fgc Using Rat Prostatitis Sera

A LnCap.fgc cell pellet was homogenized (10 gm cell pellet in 10 ml) by resuspension in PBS, 1% NP-40 and 60 µg/ml phenylmethylsulfonyl fluoride (PMSF) (Sigma, St. Louis, MO) then 10 strokes in a Dounce homogenizer. This was followed by a 30 second probe sonication and another 10 strokes in the Dounce homogenizer. The resulting slurry was centrifuged at 10,000 x G, and the supernatant filtered with a 0.45 µM filter (Amicon, Beverly, MA) then applied to a BioRad (Hercules, CA) Macro-Prep Q-20 anion exchange resin. Proteins were eluted with a 70 minute 0 to 0.8 M NaCl gradient in 20 mM tris pH 7.5 at a flow rate of 8 ml/min. Fractions were cooled, concentrated with 10 kD MWCO centriprep concentrators (Amicon) and stored at -20°C in the presence of 60 µg/ml PMSF. The ion exchange pools were then examined by electrophoresis on 4-20% tris glycine Ready-Gels (BioRad) and subsequent transfer to nitrocellulose filters. Ion exchange pools of interest were identified by ECL (Amersham International) Western analysis, using the rat sera described above in Example 3A. This analysis indicated an approximately 65 kD protein eluting at 0.08 to 0.13 M NaCl. The rat sera reactive ion exchange pool was subjected to HPLC and subsequent Western analysis to identify the protein fraction of interest. This protein was then digested for 24 hours at 25°C in 70% formic acid saturated with CNBr to cleave at methionine residues.

The resulting CNBr fragments were purified by microbore HPLC using a Vydac C18 column (Hesperia, CA), column size 1x150 mM in a Perkin Elmer/Applied Biosystems Inc. (Foster City, CA) Division Model 172 HPLC. Fractions were eluted from the column with a gradient of 0 to 60% of acetonitrile at a flow rate of 40 µl per minute. The eluent was monitored at 214 nm. The resulting fractions were loaded directly onto a Perkin Elmer/Applied Biosystems Inc. Procise 494 protein sequencer and sequenced using standard Edman chemistry from the amino terminal end. Two different peptides having the following sequences were obtained:

(a) Xaa-Ala-Lys-Lys-Phe-Leu-Asp-Ala-Glu-His-Lys-Leu-Asn-Phe-Ala (SEQ. ID No. 48); and

(b) Xaa-Xaa-Xaa-Lys-Ile-Lys-Lys-Phe-Ile-Gln-Glu-Asn-Ile-Phe-Gly,

wherein Xaa may be any amino acid (SEQ ID No. 49).

These sequences were compared to known sequences in the gene bank using databases identified above, and identified as residues 286 through 300 and 228 through 242, respectively, of probable protein disulfide isomerase ER-60 precursor, hereinafter referred to as ER-60 (Bado, R. J. et al., *Endocrinology 123*:1264-1273, 1988). This antigen is also known as phospholipase C-alpha (see PCT WO 95/08624). Residues 285 and 227 of ER-60 are methionines, consistent with the above sequences being cyanogen bromide fractions.

ER-60 is a resident endoplasmic protein with multiple biological activities, including disulfide isomerase and restricted cysteine protease activity. In particular, ER-60 has been shown to preferentially degrade calnexin, a protein involved in presentation of antigens via the Class I major histocompatability complex, or MHC, pathway. ER-60 and a related family member, ER-72, have been shown to be over-expressed in colon cancer, with truncated forms of ER-60 exhibiting increased enzymatic activity (Egea, G. et al., *J. Cell. Sci. (England) 105*:819-30, 1993). However, to the best of the inventors' knowledge, this polypeptide has not been previously shown to be present or overexpressed in human prostate. Recently, ER-60 gene expression has been correlated with induction of contact inhibition of cell proliferation (Greene, J.J. et al., *Cell. Mol. Biol. 41*:473-80, 1995). Thus, if ER-60 is also truncated and non-functional in prostatc cancer, as it is in colon cancer, the resultant loss of contact inhibition would lead to neoplastic transformation and tumor progression.

Example 4

Isolation and Characterization of Polypeptides Isolated from LnCaP.fgc Using Human Prostatitis Sera

The human prostatitis sera described above in Example 1 was used to screen the LnCaP.fgc cell line using the ion exchange techniques described above in Example 3. Reactive ion exchange pools were purified by reverse phase HPLC as described previously and the polypeptides shown in SEQ ID Nos. 50-51 were isolated utilizing cross-reactivity with said antisera as the selection criteria. Comparison of these sequences with known sequences in the gene bank using the databases described above revealed the homologies shown in Table II. However, none of these polypeptides have been previously associated with human prostate.

TABLE IV

SEQ ID No.	Database Search Identification
53	glyceraldehyde-3-phosphate-dehydrogenase
54	alpha-human fructose biphosphate aldolase
55	calreticulin
56	calreticulin
57	malate dehydrogenase
58	cystic disease fluid protein
59	cystic disease fluid protein

Example 5

Isolation and Characterization of Polypeptides from Human Seminal Fluid

Polypeptides from human seminal fluid were purified to homogeneity by anion exchange chromatography. Specifically, seminal fluid samples were diluted 1 to 10 with 0.1 mM Bis-Tris propane buffer pH 7 prior to loading on the column. The polypeptides were fractionated into pools utilizing gel profusion chromatography on a Poros (Perseptive Biosystems) 146 II Q/M anion exchange column 4.6 mm x 100 mm equilibrated in 0.01 mM Bis-Tris propane buffer pH 7.5. Proteins were eluted with a linear 0-0.5 M NaCl gradient in the above buffer. The column eluent was monitored at a wavelength of 220 nm. Individual fractions were further purified by reverse phase HLPC on a Vydac (Hesperia, CA) C18 column.

The resulting fractions were sequenced as described above in Example 3. A peptide having the following N-terminal sequence was obtained:

(c) Met-Asp-Ile-Pro-Gln-Thr-Lys-Gln-Asp-Leu-Glu-Leu-Pro-Lys-Leu (SEQ ID NO:57).

Comparison of this sequence with those of known sequences in the gene bank as described above revealed 100% identity with human placental protein 14 (PP14).

Example 6

Synthesis of Polypeptides

Polypeptides may be synthesized on an Applied Biosystems 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving

for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Corixa Corporation

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

(iii) NUMBER OF SEQUENCES: 57

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET; 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 14-MAR-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Maki, David J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 210121.424PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 622-4900
(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Arg Ala Ser Val Met Leu Leu Gly Met Met Ala Arg Gly Lys Pro
1 5 10 15

Glu Ile Val Gly Ser Asn Leu Asp Thr Leu Met Ser Ile Gly Leu Asp
20 25 30

Glu Lys Phe Pro Gln Asp Tyr Arg Leu Ala Gln Gln Val Cys His Ala
35 40 45

Ile Ala Asn Ile Ser Asp Arg Arg Lys Pro Ser Leu Gly Lys Arg His
50 55 60

Pro Pro Phe Arg Leu Pro Gln Glu His Arg Leu Phe Glu Arg Leu Arg
65 70 75 80

Glu Thr Val Thr Lys Gly Phe Val His
85

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Arg Gly Arg Phe Gly Arg Leu Gly Val Gly Gly Glu Pro His Pro
1 5 10 15

Arg Arg Asn Pro Ala Leu Pro Thr Glu Leu Ala Glu Leu Thr Pro Gln
20 25 30

Val Arg Arg Ala Ala Xaa Lys Thr Gln Arg Ser Gln Val Lys Pro Arg
35 40 45

His Arg Arg Gly Trp Pro Pro Thr Val Pro Leu Ala Gly Arg Leu Glu
50 55 60

Glu Leu Lys Thr Pro Arg Ser Pro Arg Pro Pro Glu Gln Gly Leu Asp
65 70 75 80

Pro Ser Pro Cys Ser Leu Pro Ser Pro
85

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 858 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Glu Ser Glu Pro Phe Ser His Ile Asp Pro Glu Glu Ser Glu Glu
1 5 10 15

Thr Arg Leu Leu Asn Ile Leu Gly Leu Ile Phe Lys Gly Pro Ala Ala
20 25 30

Ser Thr Gln Glu Lys Asn Pro Arg Glu Ser Thr Gly Asn Met Val Thr
35 40 45

Gly Gln Thr Val Cys Lys Asn Lys Pro Asn Met Ser Asp Pro Glu Glu
50 55 60

Ser Arg Gly Asn Asp Glu Leu Val Lys Gln Glu Met Leu Val Gln Tyr
65 70 75 80

Leu Gln Asp Ala Tyr Ser Phe Ser Arg Lys Ile Thr Glu Ala Ile Gly
85 90 95

Ile Ile Ser Lys Met Met Tyr Glu Asn Thr Thr Thr Val Val Gln Glu
100 105 110

Val Ile Glu Xaa Phe Val Met Val Phe Gln Phe Gly Val Pro Gln Ala
115 120 125

Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile Trp Ser Lys Glu Pro
130 135 140

Gly Val Arg Glu Ala Val Leu Asn Ala Tyr Arg Gln Leu Tyr Leu Asn
145 150 155 160

Pro Lys Gly Asp Ser Ala Arg Ala Lys Ala Gln Ala Leu Ile Gln Asn
165 170 175

Leu Ser Leu Leu Leu Val Asp Ala Ser Val Gly Thr Ile Gln Cys Leu
180 185 190

Glu Glu Ile Leu Cys Glu Phe Val Gln Lys Asp Glu Leu Lys Pro Ala
195 200 205

Val Thr His Leu Leu Trp Glu Arg Ala Thr Glu Lys Val Ala Cys Cys
210 215 220

Pro Leu Glu Arg Cys Ser Ser Val Met Leu Leu Gly Met Met Ala Arg
225 230 235 240

Arg Lys Pro Glu Ile Val Gly Ser Asn Leu Asp Thr Leu Met Ser Ile
245 250 255

Gly Leu Asp Glu Lys Phe Pro Gln Asp Tyr Arg Leu Ala Gln Gln Val
260 265 270

Cys His Ala Ile Ala Asn Ile Ser Asp Arg Arg Lys Pro Ser Leu Gly
275 280 285

Lys Arg His Pro Pro Phe Arg Leu Pro Gln Glu His Arg Leu Phe Glu
290 295 300

Arg Leu Arg Glu Thr Val Thr Lys Gly Phe Val His Pro Asp Pro Leu
305 310 315 320

Trp Ile Pro Phe Lys Glu Val Ala Val Thr Leu Ile Tyr Gln Leu Ala
325 330 335

Glu Gly Pro Glu Val Ile Cys Ala Gln Ile Leu Gln Gly Cys Ala Lys
340 345 350

Gln Ala Leu Glu Lys Leu Glu Glu Lys Arg Thr Ser Gln Glu Asp Pro
355 360 365

Lys Glu Ser Pro Ala Met Leu Pro Thr Phe Leu Leu Met Asn Leu Leu
370 375 380

Ser Leu Ala Gly Asp Val Ala Leu Gln Gln Leu Val His Leu Glu Gln
385 390 395 400

Ala Val Ser Gly Glu Leu Cys Arg Arg Arg Val Leu Arg Glu Glu Gln
405 410 415

Glu His Lys Thr Lys Asp Pro Lys Glu Lys Asn Thr Ser Ser Glu Thr
420 425 430

Thr Met Glu Glu Glu Leu Gly Leu Val Gly Ala Thr Ala Asp Asp Thr
435 440 445

Glu Ala Glu Leu Ile Arg Gly Ile Cys Glu Met Glu Leu Leu Asp Gly
450 455 460

Lys Gln Thr Leu Ala Ala Phe Val Pro Leu Leu Leu Lys Val Cys Asn
465 470 475 480

Asn Pro Gly Leu Tyr Ser Asn Pro Asp Leu Ser Ala Ala Ala Ser Leu
485 490 495

Ala Leu Gly Lys Phe Cys Met Ile Ser Ala Thr Phe Cys Asp Ser Gln
500 505 510

Leu Arg Leu Leu Phe Thr Met Leu Glu Lys Ser Pro Leu Pro Ile Val
515 520 525

Arg Ser Asn Leu Met Val Ala Thr Gly Asp Leu Ala Ile Arg Phe Pro
530 535 540

Asn Leu Val Asp Pro Trp Thr Pro His Leu Tyr Ala Arg Leu Arg Asp
545 550 555 560

Pro Ala Gln Gln Val Arg Lys Thr Ala Gly Leu Val Met Thr His Leu
565 570 575

Ile Leu Lys Asp Met Val Lys Val Lys Gly Gln Val Ser Glu Met Ala
580 585 590

Val Leu Leu Ile Asp Pro Glu Pro Gln Ile Ala Ala Leu Ala Lys Asn
595 600 605

Phe Phe Asn Glu Leu Ser His Lys Gly Asn Ala Ile Tyr Asn Leu Leu
610 615 620

Pro Asp Ile Ile Ser Arg Leu Ser Asp Pro Glu Leu Gly Val Glu Glu
625 630 635 640

Glu Pro Phe His Thr Ile Met Lys Gln Leu Leu Ser Tyr Ile Thr Lys
645 650 655

Asp Lys Gln Thr Glu Ser Leu Val Glu Lys Leu Cys Gln Arg Phe Arg
660 665 670

Thr Ser Arg Thr Glu Arg Gln Gln Arg Asp Leu Ala Tyr Cys Val Ser
675 680 685

Gln Leu Pro Leu Thr Glu Arg Gly Leu Arg Lys Met Leu Asp Asn Phe

690 695 700

Asp Cys Phe Gly Asp Lys Leu Ser Asp Glu Ser Ile Phe Ser Ala Phe
705 710 715 720

Leu Ser Val Val Gly Lys Leu Arg Arg Gly Ala Lys Pro Glu Gly Lys
725 730 735

Ala Ile Ile Asp Glu Phe Glu Gln Lys Leu Arg Ala Cys His Thr Arg
740 745 750

Gly Leu Asp Gly Ile Lys Glu Leu Glu Ile Gly Gln Ala Gly Ser Gln
755 760 765

Arg Ala Pro Ser Ala Lys Lys Pro Ser Thr Gly Ser Arg Tyr Gln Pro
770 775 780

Leu Ala Ser Thr Ala Ser Asp Asn Asp Phe Val Thr Pro Glu Pro Arg
785 790 795 800

Arg Thr Thr Arg Arg His Pro Asn Thr Gln Gln Arg Ala Ser Lys Lys
805 810 815

Lys Pro Lys Val Val Phe Ser Ser Asp Glu Ser Ser Glu Glu Asp Leu
820 825 830

Ser Ala Glu Met Thr Glu Asp Glu Thr Pro Lys Lys Thr Thr Pro Ile
835 840 845

Leu Arg Ala Ser Ala Arg Arg His Arg Ser
850 855

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Arg Asp Arg Leu Val Ala Ser Lys Thr Asp Gly Lys Ile Val Gln
1 5 10 15

Tyr Glu Cys Glu Gly Asp Thr Cys Gln Glu Glu Lys Ile Asp Ala Leu
20 25 30

Gln Leu Glu Tyr Ser Tyr Leu Leu Thr Ser Gln Leu Glu Ser Gln Arg
35 40 45

Ile Tyr Trp Glu Asn Lys Ile Val Arg Ile Glu Lys Asp Thr Ala Glu
50 55 60

Glu Ile Asn Asn Met Lys Thr Lys Phe Lys Glu Thr Ile Xaa Xaa Cys
65 70 75 80

Asp Asn Leu Glu His Xaa Leu Asn Asp Leu Leu Lys Glu Lys Gln Ser

85 90 95

Val Glu Arg Lys Cys Thr Gln Leu Asn Thr Lys Val Ala Lys Leu Thr
100 105 110

Asn Glu Leu Lys Glu Glu Gln Glu Met Asn Lys Cys Leu Arg Ala
115 120 125

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Arg Ala Glu Val Gln Arg Trp Arg Arg Leu Val Ala Gly Arg Arg
1 5 10 15

Arg Ala Gly Gly Asp Gly Gly Asn Ser Gly Ser Cys Ser Arg Trp Gly
20 25 30

Gly Phe Thr Ser Tyr Pro Trp Asp Arg Glu Ile
35 40

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 751 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Ala Glu Ala His Ser Asp Ser Leu Ile Asp Thr Phe Pro Glu Cys
1 5 10 15

Ser Thr Glu Gly Phe Ser Ser Asp Ser Asp Leu Val Ser Leu Thr Val
20 25 30

Asp Val Asp Ser Leu Ala Glu Leu Asp Asp Gly Met Ala Ser Asn Gln
35 40 45

Asn Ser Pro Ile Arg Thr Phe Gly Leu Asn Leu Ser Ser Asp Ser Ser
50 55 60

Ala Leu Gly Ala Val Ala Ser Asp Ser Glu Gln Ser Lys Thr Glu Glu
65 70 75 80

Glu Arg Glu Ser Arg Ser Leu Phe Pro Gly Ser Leu Lys Pro Lys Leu
85 90 95

Gly Lys Arg Asp Tyr Leu Glu Lys Ala Gly Glu Leu Ile Lys Leu Ala
100 105 110

Leu Lys Lys Glu Glu Glu Asp Asp Tyr Glu Ala Ala Ser Asp Phe Tyr
115 120 125

Arg Lys Gly Val Asp Leu Leu Leu Glu Gly Val Gln Gly Glu Ser Ser
130 135 140

Pro Thr Arg Arg Glu Ala Val Lys Arg Arg Thr Ala Glu Tyr Leu Met
145 150 155 160

Arg Ala Glu Ser Ile Ser Ser Leu Tyr Gly Lys Pro Gln Leu Asp Asp
165 170 175

Val Ser Gln Pro Pro Gly Ser Leu Ser Ser Arg Pro Leu Trp Asn Leu
180 185 190

Arg Ser Pro Ala Glu Glu Leu Lys Ala Phe Arg Val Leu Gly Val Ile
195 200 205

Asp Lys Val Leu Leu Val Met Asp Thr Arg Thr Glu His Thr Phe Ile
210 215 220

Leu Xaa Gly Leu Arg Lys Ser Ser Glu Tyr Ser Arg Asn Arg Lys Thr
225 230 235 240

Ile Xaa Pro Arg Cys Val Pro Xaa Met Val Cys Leu His Lys Tyr Ile
245 250 255

Ile Ser Glu Glu Ser Xaa Phe Leu Val Leu Gln His Ala Glu Xaa Gly
260 265 270

Lys Leu Trp Ser Tyr Ile Ser Lys Phe Leu Asn Arg Ser Pro Glu Glu
275 280 285

Ser Phe Asp Ile Lys Glu Val Lys Lys Pro Thr Leu Ala Lys Val His
290 295 300

Leu Gln Gln Pro Thr Ser Ser Pro Gln Asp Ser Ser Ser Phe Glu Ser
305 310 315 320

Arg Gly Ser Asp Gly Gly Ser Met Leu Lys Ala Leu Pro Leu Lys Ser
325 330 335

Ser Leu Thr Pro Ser Ser Gln Asp Asp Ser Asn Gln Glu Asp Asp Gly
340 345 350

Gln Asp Ser Ser Pro Lys Trp Pro Asp Ser Gly Ser Ser Ser Glu Glu
355 360 365

Glu Cys Thr Thr Ser Tyr Leu Thr Leu Cys Asn Glu Tyr Gly Gln Glu
370 375 380

Lys Ile Glu Pro Gly Ser Leu Asn Glu Glu Pro Phe Met Lys Thr Glu
385 390 395 400

Gly Asn Gly Val Asp Thr Lys Ala Ile Lys Ser Phe Pro Ala His Leu
405 410 415

Ala Ala Asp Ser Asp Ser Pro Ser Thr Gln Leu Arg Ala His Glu Leu

420 425 430

Lys Phe Phe Pro Asn Asp Asp Pro Glu Ala Val Ser Ser Pro Arg Thr
435 440 445

Ser Asp Ser Leu Ser Arg Ser Lys Asn Ser Pro Met Glu Phe Phe Arg
450 455 460

Ile Asp Ser Lys Asp Ser Ala Ser Glu Leu Leu Gly Leu Asp Phe Gly
465 470 475 480

Glu Lys Leu Tyr Ser Leu Lys Ser Glu Pro Leu Lys Pro Phe Phe Thr
485 490 495

Leu Pro Asp Gly Asp Ser Ala Ser Arg Ser Phe Asn Thr Ser Glu Ser
500 505 510

Lys Val Glu Phe Lys Ala Gln Asp Thr Ile Ser Arg Gly Ser Asp Asp
515 520 525

Ser Val Pro Val Ile Ser Phe Lys Asp Ala Ala Phe Asp Asp Val Ser
530 535 540

Gly Thr Asp Glu Gly Arg Pro Asp Leu Leu Val Asn Leu Pro Gly Glu
545 550 555 560

Leu Glu Ser Thr Arg Glu Ala Ala Ala Met Gly Pro Thr Lys Phe Thr
565 570 575

Gln Thr Asn Ile Gly Ile Ile Glu Asn Lys Leu Leu Glu Ala Pro Asp
580 585 590

Val Leu Cys Leu Arg Leu Ser Thr Glu Gln Cys Gln Ala His Glu Glu
595 600 605

Lys Gly Ile Glu Glu Leu Ser Asp Pro Ser Gly Pro Lys Ser Tyr Ser
610 615 620

Ile Thr Glu Lys His Tyr Ala Gln Glu Asp Pro Arg Met Leu Phe Val
625 630 635 640

Ala Xaa Val Asp His Ser Ser Ser Gly Asp Met Ser Leu Leu Pro Ser
645 650 655

Ser Asp Pro Lys Phe Gln Gly Leu Gly Val Val Glu Ser Xaa Val Thr
660 665 670

Ala Asn Asn Thr Glu Glu Ser Leu Phe Arg Ile Cys Ser Pro Leu Ser
675 680 685

Gly Ala Asn Glu Tyr Ile Ala Ser Thr Asp Thr Leu Lys Thr Glu Glu
690 695 700

Val Leu Leu Phe Thr Asp Gln Thr Asp Asp Leu Ala Lys Glu Glu Pro
705 710 715 720

Thr Ser Leu Phe Xaa Arg Asp Ser Glu Thr Lys Gly Glu Ser Gly Leu
725 730 735

Val Leu Glu Gly Asp Lys Glu Ile His Gln Ile Phe Glu Gly Pro
740 745 750

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Arg Gly Ser Thr Gln
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Arg Gly Ser Ser Gln Val Arg Val Lys Ser Trp Arg Gly Asp Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 271 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCGCACGAGC CTCTGTCATG CTTCTTGGCA TGATGGCACG AGGAAAGCCA GAAATTGTGG 60

GAAGCAATTT AGACACACTG ATGAGCATAG GGCTGGATGA GAAGTTTCCA CAGGACTACA 120

GGCTGGCCCA GCAGGTGTGC CATGCCATTG CCAACATCTC GGACAGGAGA AAGCCTTCTC 180

TGGGCAAACG TCACCCCCCC TTCCGGCTGC CTCAGGAACA CAGGTTGTTT GAGCGACTGC 240

GGGAGACAGT CACAAAAGGC TTTGTCCACC C 271

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGTGGATAA CCTGAGGTAG GGAGTTCGAG ACCAGCCTGA CCAACATGGA GAAACCCCAT 60

CTCTACTAAA AATAAAAAAT TAGCCGGCGT ATTGGCGTGC GCCTGTAATC CCAGCTACTC 120

AAGAGGCTGA GGCAGGAGAA TCGCCTGAAC CCAGAGGCGG AGGTTGTAGT GAGCCGAAAT 180

CACACCATTG CACTCCAGCT TGGGCAACAA TAGCGAACCT CCATCTCAAA TTAAAAAAAA 240

AATGCCTACA CGCTTCTTTA AAATGCAAGG CTTTCTCTTA AATTAGCCTA ACTGAACTGC 300

GTTGAGCTGC TTCAACTTTG GAATATATGT TTGCCAATCT CCTTGTTTTC TAATGAATAA 360

ATGTTTTTAT ATACTTTTAA AAAAAAAAAA AAAAAAACTC GAG 403

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGAGGTTTGG GCGGCTTGGC GTCGGAGGAG AGCCCCACCC GCGGAGGAAC CCAGCCTTGC 60

CAACGGAGCT GGCGGAGCTC ACTCCTCAGG TCAGGCGGGC GGCGTANAAA ACGCAGCGGA 120

GCCAGGTGAA ACCAAGGCAC CGCCGTGGCT GGCCCCCGAC AGTTCCTCTA GCCGGGAGGT 180

TGGAGGAGCT GAAAACGCCG CGGAGCCCTC GGCCGCCCGA GCAGGGGCTG GACCCCAGCC 240

CTTGCAGCCT CCCTTCTCCT GGCACCCAAG TGCAGTCCTG GCTGCAGAAG GGGCCGCGGG 300

CGCACTGAGT TTCCAACCTC CGTTCAGCCT GTCTGTCTCA GGGTGCAGCC TTAATGAGAG 360

GTGATTCCTA AGCTGCTGGG AACCTGAGGT TGTCAAAGGG GCGGCAGGAA ATGGACAGCA 420

GTATAAAACC CAGAAGCAGA ACTTGAAGGT TAAACCACTA GCCCATTTCA CAGAATGTTT 480

CATCCATTTG TGGACCAAAA GATGGAGTTG GTTTTTATTT TTAAAAAGAT AATGTTAATG 540

ATCTGATACC ACTACAAATA TTTACGTGAG AAGATTCATG GACTTGTCTT TTGGTTGGAC 600

TGTCACTCAT TTCTGAAAGT TTCTTCAGCC ACAATTTCTA TTTGAAAATT CAAGTATCAA 660

AGGATACCAG GTTTAGAATG GTATAATGAT GTATTTTGTC TGAGGACTGC AAATTTTATA 720

GAGACCACAG TTGGATTCCA GTGATATTCT GCAATCAAAG TGATTTGATA AACCTAATTT 780

TGAAGCATTT TATATTTATA AGCGACATCA AAAGATGGGA GAAAAAAATG GCGATGCAAA 840

AACTTTCTGG ATGGAGCTAG AAGATGATGG AAAAGTGGAC TTCATTTTTG AACAAGTACA 900

AAATGTGCTG CAGTCACTGA AACAAAAGAT CAAAGATGGG TCTGCCACCA ATAAAGAATA 960

CATCCAAGCA ATGATTCTAG TGAATGAAGC AACTATAATT AACAGTTCAA CATCAATAAA 1020

GGATCCTATG CCTGTGACTC AGAAGGAACA GGAAAACAAA TCCAATGCAT TTCCCTCTAC 1080

ATCATGTGAA AACTCCTTTC CAGAAGACTG TACATTTCTA ACAACAGGAA ATAAGGAAAT 1140

TCTCTCTCTT GAAGATAAAG TTGTAGACTT TAGAGAAAAA GACTCATCTT CGAATTTATC 1200

TTACCAAAGT CATGACTGCT CTGGTGCTTG TCTGATGAAA ATGCCACTGA ACTTGAAGGG 1260

AGAAAACCCT CTGCAGCTGC CAATCAAATG TCACTTCCAA AGACGACATG CAAAGACAAA 1320

CTCTCATTCT TCAGCACTCC ACGTGAGTTA TAAAACCCCT TGTGGAAGGA GTCTACGAAA 1380

CGTGGAGGAA GTTTTTCGTT ACCTGCTTGA GACAGAGTGT AACTTTTTAT TTACAGATAA 1440

CTTTTCTTTC AATACCTATG TTCAGTTGGC TCGGAATTAC CCAAAGCAAA AAGAAGTTGT 1500

TTCTGATGTG GATATTAGCA ATGGAGTGGA ATCAGTGCCC ATTTCTTTCT GTAATGAAAT 1560

TGACAGTAGA AAGCTCCCAC AGTTTAAGTA CAGAAAGACT GTGTGGCCTC GAGCATATAA 1620

TCTAACCAAC TTTTCCAGCA TGTTTACTGA TTCCTGTGAC TGCTCTGAGG GCTGCATAGA 1680

CATAACAAAA TGTGCATGTC TTCAACTGAC AGCAAGGAAT GCCAAAACTT CCCCCTTGTC 1740

AAGTGACAAA ATAACCACTG GATATAAATA TAAAAGACTA CAGAGACAGA TTCCTACTGC 1800

CATTTATGAA TGCAGCCTTT TGTGCAAATG TAATCGACAA TTGTGTCAAA ACCGAGTTGT 1860

CCAACATGGT CCTCAAGTGA GGTTACAGGT GTTCAAAACT GAGCAGAAGG GATGGGGTGT 1920

ACGCTGTCTA GATGACATTG ACAGAGGGAC ATTTGTTTGC ATTTATTCAG GAAGATTACT 1980

AAGCAGAGCT AACACTGAAA AATCTTATGG TATTGATGAA AACGGGAGAG ATGAGAATAC 2040

TATGAAAAAT ATATTTTCAA AAAAGAGGAA ATTAGAAGTT GCATGTTCAG ATTGTGAAGT 2100

TGAAGTTCTC CCATTAGGAT TGGAAACACA TCCTAGAACT GCTAAAACTG AGAAATGTCC 2160

ACCAAAGTTC AGTAATAATC CCAAGGAGCT TACTATGGAA ACGAAATATG ATAATATTTC 2220

AAGAATTCAG TATCATTCAG TTATTAGAGA TCCTGAATCC AAGACAGCCA TTTTTC 2276

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3114 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAGGAGTCCG AACCCTTCAG TCATATAGAC CCAGAGGAGT CAGAGGAGAC CAGGCTCTTG 60

AATATCTTAG GACTTATCTT CAAAGGCCCA GCAGCTTCCA CACAAGAAAA GAATCCCCGG 120

GAGTCTACAG GAAACATGGT CACAGGACAG ACTGTCTGTA AAAATAAACC CAATATGTCG 180

GATCCTGAGG AATCCAGGGG AAATGATGAA CTAGTGAAGC AGGAGATGCT GGTACAGTAT 240

CTGCAGGATG CCTACAGCTT CTCCCGGAAG ATTACAGAGG CCATTGGCAT CATCAGCAAG 300

ATGATGTATG AAAACACAAC TACAGTGGTG CAGGAGGTGA TTGAATNCTT TGTGATGGTC 360

TTCCAATTTG GGGTACCCCA GGCCCTGTTT GGGGTGCGCC GTATGCTGCC TCTCATCTGG 420

TCTAAGGAGC CTGGTGTCCG GGAAGCCGTG CTTAATGCCT ACCGCCAACT CTACCTCAAC 480

CCCAAAGGGG ACTCTGCCAG AGCCAAGGCC CAGGCTTTGA TTCAGAATCT CTCTCTGCTG 540

CTAGTGGATG CCTCGGTTGG GACCATTCAG TGTCTTGAGG AAATTCTCTG TGAGTTTGTG 600

CAGAAGGATG AGTTGAAACC AGCAGTGACC CATCTGCTGT GGGAGCGGGC CACCGAGAAG 660

GTCGCCTGCT GTCCTCTGGA GCGCTGTTCC TCTGTCATGC TTCTTGGCAT GATGGCACGA 720

AGAAAGCCAG AAATTGTGGG AAGCAATTTA GACACACTGA TGAGCATAGG GCTGGATGAG 780

AAGTTTCCAC AGGACTACAG GCTGGCCCAG CAGGTGTGCC ATGCCATTGC CAACATCTCG 840

GACAGGAGAA AGCCTTCTCT GGGCAAACGT CACCCCCCCT TCCGGCTGCC TCAGGAACAC 900

AGGTTGTTTG AGCGACTGCG GGAGACAGTC ACAAAAGGCT TTGTCCACCC AGACCCACTC 960

TGGATCCCAT TCAAAGAGGT GGCAGTGACC CTCATTTACC AACTGGCAGA GGGCCCCGAA 1020

GTGATCTGTG CCCAGATATT GCAGGGCTGT GCAAAACAGG CCCTGGAGAA GCTAGAAGAG 1080

AAGAGAACCA GTCAGGAGGA CCCGAAGGAG TCCCCCGCAA TGCTCCCCAC TTTCCTGTTG 1140

ATGAACCTGC TGTCCCTGGC TGGGGATGTG GCTCTGCAGC AGCTGGTCCA CTTGGAGCAG 1200

GCAGTGAGTG GAGAGCTCTG CCGGCGCCGA GTTCTCCGGG AAGAACAGGA GCACAAGACC 1260

AAAGATCCCA AGGAGAAGAA TACGAGCTCT GAGACCACCA TGGAGGAGGA GCTGGGGCTG 1320

GTTGGGGCAA CAGCAGATGA CACAGAGGCA GAACTAATCC GTGGCATCTG CGAGATGGAA 1380

CTGTTGGATG GCAAACAGAC ACTGGCTGCC TTTGTTCCAC TCTTGCTTAA AGTCTGTAAC 1440

AACCCAGGCC TCTATAGCAA CCCAGACCTC TCTGCAGCTG CTTCACTTGC CCTTGGCAAG 1500

TTCTGCATGA TCAGTGCCAC TTTCTGCGAC TCCCAGCTTC GTCTTCTGTT CACCATGCTG 1560

GAAAAGTCTC CACTTCCCAT TGTCCGGTCT AACCTCATGG TTGCCACTGG GGATCTGGCC 1620

ATCCGCTTTC CCAATCTGGT GGACCCCTGG ACTCCTCATC TGTATGCTCG CCTCCGGGAC 1680

CCTGCTCAGC AAGTGCGGAA AACAGCGGGG CTGGTGATGA CCCACCTGAT CCTCAAGGAC 1740

ATGGTGAAGG TGAAGGGGCA GGTCAGTGAG ATGGCGGTGC TGCTCATCGA CCCCGAGCCT 1800

CAGATTGCTG CCCTGGCCAA GAACTTCTTC AATGAGCTCT CCCACAAGGG CAACGCAATC 1860

TATAATCTCC TTCCAGATAT CATCAGCCGC CTGTCAGACC CCGAGCTGGG GGTGGAGGAA 1920

GAGCCTTTCC ACACCATCAT GAAACAGCTC CTCTCCTACA TCACCAAGGA CAAGCAGACA 1980

GAGAGCCTGG TGGAAAAGCT GTGTCAGCGG TTCCGCACAT CCCGAACTGA GCGGCAGCAG 2040

CGAGACCTGG CCTACTGTGT GTCACAGCTG CCCCTCACAG AGCGAGGCCT CCGTAAGATG 2100

CTTGACAATT TTGACTGTTT TGGAGACAAA CTGTCAGATG AGTCCATCTT CAGTGCTTTT 2160

TTGTCAGTTG TGGGCAAGCT GCGACGTGGG GCCAAGCCTG AGGGCAAGGC TATAATAGAT 2220

GAATTTGAGC AGAAGCTTCG GGCCTGTCAT ACCAGAGGTT TGGATGGAAT CAAGGAGCTT 2280

GAGATTGGCC AAGCAGGTAG CCAGAGAGCG CCATCAGCCA AGAAACCATC CACTGGTTCT 2340

AGGTACCAGC CTCTGGCTTC TACAGCCTCA GACAATGACT TTGTCACACC AGAGCCCCGC 2400

CGTACTACCC GTCGGCATCC AAACACCCAG CAGCGAGCTT CCAAAAAGAA ACCCAAAGTT 2460

GTCTTCTCAA GTGATGAGTC CAGTGAGGAA GATCTTTCAG CAGAGATGAC AGAAGACGAG 2520

ACACCCAAGA AAACAACTCC CATTCTCAGA GCATCGGCTC GCAGGCACAG ATCCTAGGAA 2580

GTCTGTTCCT GTCCTCCCTG TGCAGGGTAT CCTGTAGGGT GACCTGGAAT TCGAATTCTG 2640

TTTCCCTTGT AAAATATTTG TCTGTCTCTT TTTTTTAAAA AAAAAAAAGG CCGGGCACTG 2700

TGGCTCACGC CTGTAATCCC AGCACTTTGC GATACCAAGG CGGGTGGATA ACCTGAGGTA 2760

GGGAGTTCGA GACCAGCCTG ACCAACATGG AGAAACCCCA TCTCTACTAA AAATAAAAAA 2820

TTAGCCGGGC GTATTGGCGT GCGCCTGTAA TCCCAGCTAC TCAAGAGGCT GAGGCAGGAG 2880

AATCGCCTGA ACCCAGAGGC GGAGGTTGTA GTGAGCCGAA ATCACACCAT TGCACTCCAG 2940

CTTGGGCAAC AATAGCGAAC CTCCATCTCA AATTAAAAAA AAAATGCCTA CACGCTCTTT 3000

AAAATGCAAG GCTTTCTCTT AAATTAGCCT AACTGAACTG CGTTGAGCTG CTTCAACTTT 3060

GGAATATATG TTTGCCAATC TCCTTGTTTT CTAATGAATA AATGTTTTTA TATA 3114

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGGCACGAGA TCGACTGGTT GCAAGTAAAA CAGATGGAAA AATAGTACAG TATGAATGTG 60

AGGGGGATAC TTGCCAGGAA GAGAAAATAG ATGCCTTACA GTTAGAGTAT TCATATTTAC 120

TAACAAGCCA GCTGGAATCT CAGCGAATCT ACTGGGAAAA CAAGATAGTT CGGATAGAGA 180

AGGACACAGC AGAGGAAATT AACAACATGA AGACCAAGTT TAAAGAAACA ATTGAGAAGT 240

GTGATAATCT AGAGCACAAA CTAAATGATC TCCTAAAAGA AAAGCAGTCT GTGGAAAGAA 300

AGTGCACTCA GCTAAACACA AAAGTGGCCA AACTCACCAA CGAGCTCAAA GAGGAGCAGG 360

AAATGAACAA GTGTTTGCGA GCCAACCAAG TCCTCCTGCA GAACAAGCTA AAAGAGGAGG 420

AGAGGGTGCT GAAGGAGACC TGTGACCAAA AAGATCTGCA GATCACCGAG ATCCAGGAGC 480

AGCTGCGTGA CGTCATGTTC TACCTGGAGA CACAGCAGAA GATCAACCAT CTGCCTGCCG 540

AGACCCGGCA GGAAATCCAG GAGGGACAGA TCAACATCGC CATGGCCTCG GCCTCGAGCC 600

CTGCCTCTTC GGGGGGCAGT GGGAAGTTGC CCTCCAGGAA GGGCCGCAGC AAGAGGGGCA 660

AGTGACCTTC AGAGCAACAG ACATCCCTGA GACTGTTCTC CCTGACACTG TGAGAGTGTG 720

CTGGGACCTT CAGCTAAATG TGAGGGTGGG CCCTAATAAG TACAAGTGAG GATCAAGCCA 780

CAGTTGTTTG GCTCTTTCAT TTGCTAGTGT GTGATGTANT GAATGTAAAG GGTGCTGACT 840

GGAGAGCTGA TAGAAAGGCG CTGCGTTCGA AAAGGTCTTA ANAGTTCACT AACCTCACAT 900

TCTAATGACC ATTTTGCCTT CCTGCTTGGT AGAAGCCCCA ACTCTGCTGT GCATTTTTCC 960

ATTGTATTTA TGGAGTTGGC GTATTTGACA TTCAGTTCTG GGGTAGGTTT AAGATGTTAA 1020

GTTATTTCTT GTAACCTCAA AGGTAAGGTT ATCTAGCACT AAAGCACCAA ACCTCTCTGA 1080

GGGCATAACA GCTGCTTTAA AGAGAGGTTT CCATTGGCTA TTAAGGAGTT ATGAAAACTC 1140

CCTAGCAATA GTGTCATATC ATTATCATCT CCCCCTTCCT CTGGGGAGTG GAAGAATTGC 1200

TTGAATGTTA TCTGAAAAGA GGCCTGGTAG TAAACCAGGC CCTGGCTCTT TACCAGCAGT 1260

CATCTCTTCT TGCTCTGGGG CCAGCCAGGA AAAACAAACA ACCCGGGGCA CATTGGGTAG 1320

ACTCAGTGTA GGAAAAATGG TGGCAGCTCC ACTGTTTATT TTTGGTGACT TCGTACGTCA 1380

TTATGAACCG CAATTAAGGA GGAGGCTTAA TGGCTGTTCC CAAACTCAAA TCTCAGAGTG 1440

GGTATCCTAG CATCTAGCAA NACTGAGTGG GGAGATTTCT CATCCGTGTG AAAATGTAGA 1500

GTGAGGCCTC TGACTAGCTN ATTGTGTATT TTGTTGGGTT TAGTATTTTC TAAATGTTTA 1560

CAAAATATTG GGCTGCATGT TCAGGTTGCA GCTANAGGGA GCTTGGGCAN ATTTTCAATT 1620

ACGCTTTCAA GATATAACCA AAAGCTGTTT CTAAATCCTA AAATTAGAAT TTCAACAGAN 1680

CCCCCTTTAG AACAGTCATA TAACGCTTGT GTGGGCCAAC AGANGGGCTG TGTACTCTCT 1740

CTGGAACCAT AAATGTCAAA TAATTTATAA CCTGCANTAA TTGAGCAACT TAAATAA 1797

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 720 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TAATCACCAT CTGTTTTTGT GGGATGTGCT GCAGCATTTC CCAAAAAACT TNACGTGTAA 60

TGTTGCAAAA TGAATGTACT CAGACATTNT TAATTTTTAC TTAGGGCAGA CCAACTCTTT 120

GAGTCTCTCT TGGACTTATA TATACAGATA TCTTAAGAGT GGGAATGTAA AGCATAACCT 180

AATTNTCTTT CCTATAGAGA TTCTATTTTA TTTAAAATNT ATTTNTACAC TAGTTAGAAT 240

CCTGCTGTTT TGGCCAAGTA CTTGTCTTGC ATGTCTGACC TTGCAGAAGC TGGGGTGGAT 300

CATAGCATAC TAATGAAGAG AATTAGAAGT AGTTTACAAA GCTCGCTCAC TCCTCATTTC 360

TCTGTGATCC CTTCTATCCA GTGGCCCCAC CACCACCTGG GAAAACAGAT TTTTCAGTAC 420

AGGTGGGATA AATGCTCTGA AAGGCTGTGC CCAGAGGAAT GAGCAAATAG GCAAGTGTTT 480

CCAAACTACT TGGAGGTTTA CAAAAAATAT GTCCCAGAAA AAAAAAAAAT CTTACCAAGA 540

TACGTAAAGA AAAAAAAATT TTTTTTTAAA CAGTCAAAGA GTCATGTTTG AATTTCACAA 600

AATCACATCA GACAGAAGTT GTTTTCTTCA GGAGGGAAAT GAACCACTTA ATATACCCAT 660

ACTACCTTGA ACAATGAAAT TGAATTAAAA TAGCCAAACT TTGAAAAAAA AAAAAAAAAA 720

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1996 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGAAGTGCA GCGGTGGCGG CGGCTGGTTG CGGGCCGGCG GCGGGCTGGC GGAGATGGAG 60

GTAACTCAGG ATCTTGTTCA AGATGGGGTG GCTTCACCAG CTACCCCTGG GACCGGGAAA 120

TCTAAGCTGG AAACATTGCC CAAAGAAGAC CTCATCAAGT TTGCCAAGAA ACAGATGATG 180

CTAATACAGA AAGCTAAATC AAGGTGTACA GAATTGGAGA AAGAAATTGA AGAACTCAGA 240

TCAAAACCTG TTACTGAAGG AACTGGTGAT ATTATTAAGG CATTAACTGA ACGTCTGGAT 300

GCTCTTCTTC TGGAAAAAGC AGAGACTGAG CAACAGTGTC TTTCTCTGAA AAAGGAAAAT 360

ATAAAAATGA AGCAAGAGGT TGAGGATTCT GTAACAAAGA TGGGAGATGC ACATAAGGAG 420

TTGGAACAAT CACATATAAA CTATGTGAAA GAAATTGAAA ATTTGAAAAA TGAGTTGATG 480

GCAGTACGTT CCAAATACAG TGAAGACAAA GCTAACTTAC AAAAGCAGCT GGAAGAACAA 540

TGAATACGCA ATTAGAACTT TCAGAACAAC TTAAATTTCA GAACAACTCT GAAGATAATG 600

TTAAAAAACT ACAAGAAGAG ATTGAGAAAA TTAGGCCAGG CTTTGAGGAG CAAATTTTAT 660

ATCTGCAAAA GCAATTAGAC GCTACCACTG ATGAAAAGAA GGAAACAGTT ACTCAACTCC 720

AAAATATCAT TGAGGCTAAT TCTCAGCATT ACCAAAAAAA TATTAATAGT TTGCAGGAAG 780

AGCTTTTACA GTTGAAAGCT ATACACCAAG AAGAGGTGAA AGAGTTGATG TGCCAGATTG 840

AAGCATCAGC TAAGGAACAT GAAGCAGAGA TAAATAAGTT GAACGAGCTA AAAGAGAACT 900

TAGTAAAACA ATGTGAGGCA AGTGAAAAGA ACATCCAGAA GAAATATGAA TGTGAGTTAG 960

AAAATTTAAG GAAAGCCACC TCAAATGCAA ACCAAGACAA TCAGATATGT TCTATTCTCT 1020

TGCAAGAAAA TACATTTGTA GAACAAGTAG TAAATGAAAA AGTCAAACAC TTAGAAGATA 1080

CCTTAAAAGA ACTTGAATCT CAACACAGTA TCTTAAAAGA TGAGGTAACT TATATGAATA 1140

ATCTTAAGTT AAAACTTGAA ATGGATGCTC AACATATAAA GGATGAGTTT TTTCATGAAC 1200

GGGAAGACTT AGAGTTTAAA ATTAATGAAT TATTACTAGC TAAAGAAGAA CAGGGCTGTG 1260

TAATTGAAAA ATTAAAATCT GAGCTAGCAG GTTTAAATAA ACAGTTTTGC TATACTGTAG 1320

AACAGCATAA CAGAGAAGTA CAGAGTCTTA AGGAACAACA TCAAAAAGAA ATATCAGAAC 1380

TAAATGAGAC ATTTTTGTCA GATTCAGAAA AAGAAAAATT AACATTAATG TTTGAAATAC 1440

AGGGTCTTAA GGAACAGTGT GAAAACCTAC AGCAAGAAAA GCAAGAAGCA ATTTTAAATT 1500

ATGAGAGTTT ACGAGAGATT ATGGAAATTT TACAAACAGA ACTGGGGGAA TCTGCTGGAA 1560

AAATAAGTCA AGAGTTCGAA TCAATGAAGC AACAGCAAGC ATCTGATGTT CATGAACTGC 1620

AGCAGAAGCT CAGAACTGCT TTTACTGAAA AAGATGCCCT TCTCGAAACT GTGAATCGCC 1680

TCCAGGGAGA AAATGAAAAG TTACTATCTC AACAAGAATT GGTACCAGAA CTTGAAAATA 1740

CCATAAAGAA CCTTCAAGAA AAGAATGGAG TATACTTACT TAGTCTCAGT CAAAGAGATA 1800

CCATGTTAAA AGAATTAGAA GGAAAGATAA ATTCTCTTAC TGAGGAAAAA GATGATTTTA 1860

TAAATAAACT GAAAAATTCC CATGAAGAAA TGGATAATTT CCATAAGAAA TGTGAAAGGG 1920

AAGAAAGATT GATTCTTGAA CTTGGGAAGA AAGTAGAGCA AACTATCCAG TACAACAGTG 1980

AACTAGAACA AAAGGT 1996

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3642 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCCTGCTGA AGCTCACTCA GATTCCCTCA TTGATACCTT TCCTGAGTGT AGTACGGAAG 60

GCTTCTCCAG TGACAGTGAT CTGGTATCTC TTACTGTTGA TGTGGATTCT CTTGCTGAGT 120

TAGATGATGG AATGGCTTCC AATCAAAATT CTCCCATTAG AACTTTTGGT CTCAATCTTT 180

CTTCGGATTC TTCAGCACTA GGGGCTGTTG CTTCTGACAG TGAACAGAGC AAAACAGAAG 240

AAGAACGGGA AAGTCGTAGC CTCTTTCCTG GCAGTTTAAA GCCGAAGCTT GGCAAGAGAG 300

ATTATTTGGA GAAAGCAGGA GAATTAATAA AGCTGGCTTT AAAAAAGGAA GAAGAAGACG 360

ACTATGAAGC TGCTTCTGAT TTTTATAGGA AGGGAGTTGA TTTACTCCTA GAAGGTGTTC 420

AAGGAGAGTC AAGCCCTACC CGTCGAGAAG CTGTGAAGAG AAGAACAGCC GAGTACCTCA 480

TGCGGGCAGA AAGTATCTCT AGTCTTTATG GGAAACCTCA GCTTGATGAT GTATCTCAGC 540

CTCCAGGATC ACTAAGTTCA AGGCCCCTTT GGAACCTAAG GAGCCCTGCC GAGGAGCTGA 600

AGGCCTTCAG AGTCCTTGGG GTGATTGACA AGGTTTTACT TGTAATGGAC ACAAGGACAG 660

AACACACTTT CATTTTAANA GGTCTAAGGA AAAGCAGTGA ATACAGCAGG AACAGAAAGA 720

CCATCCNCCC CCGCTGTGTG CCCANCATGG TGTGTCTGCA TAAGTACATC ATCTCTGAAG 780

AGTCANTATT TCTTGTGCTG CAGCATGCGG AANGTGGCAA ACTGTGGTCA TATATCAGTA 840

AATTTCTAAA CAGAAGTCCT GAAGAAAGCT TTGACATCAA GGAAGTGAAA AAACCTACAC 900

TTGCAAAAGT TCACCTGCAG CAGCCAACTT CTAGTCCTCA GGACAGCAGT AGCTTTGAAT 960

CCAGAGGAAG TGATGGTGGA AGCATGCTTA AAGCTCTGCC TTTGAAGAGT AGTCTTACTC 1020

CAAGTTCTCA AGATGACAGC AACCAGGAAG ATGATGGCCA AGATAGCTCT CCAAAGTGGC 1080

CAGATTCTGG TTCAAGTTCA GAAGAAGAAT GTACTACTAG TTATTTAACA TTATGCAATG 1140

AATATGGGCA AGAAAAGATT GAACCAGGGT CTTTGAATGA GGAGCCCTTC ATGAAGACTG 1200

AAGGGAATGG TGTTGATACA AAAGCTATTA AAAGCTTCCC AGCACACCTT GCTGCTGACA 1260

GTGACAGCCC CAGCACACAG CTGAGAGCTC ACGAGCTGAA GTTCTTCCCC AACGATGACC 1320

CAGAAGCAGT TAGTTCTCCA AGAACATCAG ATTCCCTCAG TAGATCAAAA AATAGCCCCA 1380

TGGAATTCTT TAGGATAGAC AGTAAGGATA GCGCAAGTGA ACTCCTGGGA CTTGACTTTG 1440

GAGAAAAATT GTATAGTCTA AAATCAGAAC CTTTGAAACC ATTCTTTACT CTTCCAGATG 1500

GAGACAGTGC TTCTAGGAGT TTTAATACTA GTGAAAGCAA GGTAGAGTTT AAAGCTCAGC 1560

ACACCATTAG CAGGGGCTCA GATGACTCAG TGCCAGTTAT TTCATTTAAA GATGCTGCTT 1620

TTGATGATGT CAGTGGTACT GATGAAGGAA GACCTGATCT TCTTGTAAAT TTACCTGGTG 1680

AATTGGAGTC AACAAGAGAA GCTGCAGCAA TGGGACCTAC TAAGTTTACA CAAACTAATA 1740

TAGGGATAAT AGAAAATAAA CTCTTGGAAG CCCCTGATGT TTTATGCCTC AGGCTTAGTA 1800

CTGAACAATG CCAAGCACAT GAGGAGAAAG GCATAGAGGA ACTGAGTGAT CCCTCTGGGC 1860

CCAAATCCTA TAGTATAACA GAGAAACACT ATGCACAGGA GGATCCCAGG ATGTTATTTG 1920

TAGCANCTGT TGATCATAGT AGTTCAGGAG ATATGTCTTT GTTACCCAGC TCAGATCCTA 1980

AGTTTCAAGG ACTTGGAGTG GTTGAGTCAN CAGTAACTGC AAACAACACA GAAGAAAGCT 2040

TATTCCGTAT TTGTAGTCCA CTCTCAGGTG CTAATGAATA TATTGCAAGC ACAGACACTT 2100

TAAAAACAGA AGAAGTATTG CTGTTTACAG ATCAGACTGA TGATTTGGCT AAAGAGGAAC 2160

CAACTTCTTT ATTCCANAGA GACTCTGAGA CTAAGGGTGA AAGTGGTTTA GTGCTAGAAG 2220

GAGACAAGGA AATACATCAG ATTTTTGAAG GACCTTGATA AAAAATTAGC ACTANCCTCC 2280

AGGTTTTACA TCCCAGAGGG CTGCATTCAA AGNTGGGCAG CTGAAATGGT GGTAGCCCTT 2340

NGATGCTTTA ACATAGAGAG GGAATTGTGT GCCGCGATTG AACCCAAACA ANATNTTATT 2400

GAATGATAGA GGACACATTC AGNTAACGTA TTTTAGCAGG TGGAGTGAGG TTGAAGATTC 2460

CTGTGACAGC GATGCCATAG AGAGAATGTA CTGTGCCCCA GAGGTTGGAG CAATCACTGA 2520

AGAAACTGAA GCCTGTGATT GGTGGAGTTT GGGTGCTGTC CTCTTTGAAC TTNTCACTGG 2580

CAAGACTCTG GTTGAATGCC ATCCAGCAGG AATAAATACT CACACTACTT TGAACATGCC 2640

AGAATGTGTC TCTGAAGAGG CTCGCTCACT CATTCAACAG CTCTTGCAGT TCAATCCTCT 2700

GGAACGACTT GGTGCTGGAG TTGCTGGTGT TGAAGATATC AAATCTCATC CATTTTTTAC 2760

CCCTGTGGAT TGGGCAGAAC TGATGAGATG AACGTAATGC AGGGTTATCT TCACACATTC 2820

TGATCTTCTC TGTGACAGGC ATCTCCAGCA CTGAGGCACC TCTGACTCAC AGTTACTTAT 2880

GGAGCACCAA AGCATTTGGA TAAGGACCGT TATAGGAAAT GGGGGGGAAA TGGCTAAAAG 2940

AGAACAATTT GTTTACAATT ACAAGATATT AGCTAATTGT GCCAGGGGCT GTTATATACA 3000

TATATACACA ACCAAGGTGT GATCTGAATT TAATCCACAT TTGGTGTTGC AGATGAGTTG 3060

TAAAGCCAAC TGAAAGAGTT CCTTCAAGAA GTTCCTCTGA TAGGAAGCTA GAAGTGTAGA 3120

ATGAAGTTTT ACTTGACAGA AGGACCTTTA CATGGCAGCT AACAGTGCTT TTTGCTGACC 3180

AGGATTGGTT TATATGATTA AATTAATATT TGCTTAATAA TACACTAAAA GTATATGAAC 3240

AATGTCATCA ATGAAACTTA AAAGCGAGAA AAAAGAATAT ACACATAATT TCTGACGGAA 3300

AACCTGTACC CTGATGCTGT ATAATGTATG TTGAATGTGG TCCCAGATTA TTTCTGTAAG 3360

AAGACACTCC ATGTTGTCAG CTTTGTACTC TTTGTTGATA CTGCTTATTT AGAGAAGGGT 3420

TCATATAAAC ACTCACTCTG TGTCTTCAAC AGCATCTTTC TTTCCCCATC TTTCTATTTT 3480

CTGCACCCTC TGCTTGTTCC CTCATATTCT GTTCTTCCGA CTCCTGCTAA CACACATGCA 3540

ACAAAAAAGG GAAGGGAGTG CTTATTTCCC TTTGTGTAAG GACTAAGAAA TCATGATATC 3600

AAATAAACAT GGTGAAACAT TNANAAAAAA AAAAAAAAAA AA 3642

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1397 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTTCAACTCA ATAGAAGATG ACGTTTGCCA GCTAGTGTAT GTGGAAAGAG CTGAAGTGCT 60

CAAATCTGAA GATGGCGCCA GCCTCCCAGT GATGGACCTG ACTGAACTCC CCAAGTGCAC 120

GGTGTGTCTG GAGCGCATGG ACGAGTCTGT GAATGGCATC CTCACAACGT TATGTAACCA 180

CATCTTCCAC AGCCAGTGTC TACAGCGCTG GGACGATACC ACGTGTCCTG TTTGCCGGTA 240

CTGTCAAACG CCCGAGCCAG TAGAAGAAAA TAAGTGTTTT GAGTGTGGTG TTCAGGAAAA 300

TCTTTGGATT TGTTTAATAT GCGGCCACAT AGGATGTGGA CGGTATGTCA GTCGACATGC 360

TTATAAGCAC TTTGAGGAAA CGCAGCACAC GTATGCCATG CAGCTTACCA ACCATCGAGT 420

CTGGGACTAT GCTGGAGATA ACTATGTTCA TCGACTGGTT GCAAGTAAAA CAGATGGAAA 480

AATAGTACAG TATGAATGTG AGGGGGATAC TTGCCAGGAA GAGAAAATAG ATGCCTTACA 540

GTTAGAGTAT TCATATTTAC TAACAAGCCA GCTGGAATCT CAGCGAATCT ACTGGGAAAA 600

CAAGATAGTT CGGATAGAGA AGGACACAGC AGAGGAAATT AACAACATGA AGACCAAGTT 660

TAAAGAAACA ATTGAGAAGT GTGATAATCT AGAGCACAAA CTAAATGATC TCCTAAAAGA 720

AAAGCAGTCT GTGGAAAGAA AGTGCACTCA GCTAAACACA AAAGTGGCCA AACTCACCAA 780

CGAGCTCAAA GAGGAGCAGG AAATGAACAA GTGTTTGCGA GCCAACCAAG TCCTCCTGCA 840

GAACAAGCTA AAAGAGGAGG AGAGGGTGCT GAAGGAGACC TGTGACCAAA AAGATCTGCA 900

GATCACCGAG ATCCAGGAGC AGCTGCGTGA CGTCATGTTC TACCTGGAGA CACAGCAGAA 960

AGATCAACCA TCTGCCTGCC GAGACCCGGC AGGAAATCCA GGAGGGACAG ATCAACATCG 1020

CCATGGCCTC GGCCTCGAGC CCTGCCTCTT CGGGGGGCAG TGGGAAGTTG CCCTCCAGGA 1080

AGGGCCGCAG CAAGAGGGGC AAGTGACCTT CAGAGCAACA GACATCCCTG AGACTGTTCT 1140

CCCTGACACT GTGAGAGTGT GCTGGGACCT TCAGCTAAAT GTGAGGGTGG GCCCTAATAA 1200

GTACAAGTGA GCATCAAGCC ACAGTTGTTT GGCTCTTTCA TTTGCTAGTG TGTGATGTAG 1260

TGAATGTAAA GGGTGCTGAC TGGAGAGCTG ATAGAAAGGC GCTGCGTTCG AAAAGGTCTT 1320

AAGAGTTCAC TAACCTCACA TTCTAATGAC CANTTTGCCT TCCTGCTTGG TAGAAGCCCC 1380

ACACTCTGCT GTGCATT 1397

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 800 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGTAATTGA GCANACTTAA AATAAGACCT GTGTTGGAAT TTAGTTTCCT CTGAAGAGGT 60

AGAGGGATAG GTTAGTAAGA TGTATTGTTA AACAACAGGT TTTAGTTTTT GCTTTTATAA 120

TTAGCCACAG GTTTTCAAAT GATCACATTT CAGAATAGGT TTTTAGCCTG TAATTAGGCC 180

TCATCCCCTT TGACCTAAAT GTCTTACATG TTACTTGTTA GCACATCAAC TGTATCACTA 240

ATCACCATCT GNTTTTGTGG GATGTGCTGC AGCATTTCCC AAAAAACTTT ACGTGTAATG 300

TTGCAAAATG AATGTACTCA GACATTCTTA ATTTTTACTT AGGGCAGACC AACTCTTTGA 360

GTCTCTCTTG GACTTATATA TACAGATATC TTAAGAGTGG GAATGTAAAG CATAACCTAA 420

TTCTCTTTCC TATAGAGATT CTATTTTATT TAAAATCTAT TTTTACACTA GTTAGAATCC 480

TGCTGTTTTG GCCAAGTACT TGTCTTGCAT GTCTGACCTT GCAGAAGCTG GGGTGGATCA 540

TAGCATACTA ATGAAGAGAA TTAGAAGTAG TTTACAAAGC TCGCTCACTC CTCATTTCTC 600

TGTGATCCCT TCTATCCAGT GGCCCCACCA CCACCTGGGA AAACAGATTT TTCAGTACAG 660

GTGGGATAAA TGCTCTGAAA GGCTGTGCCC AGAGGAATGA GCAAATAGGC AAGTGTTTCC 720

AAACTACTTG GAGGTTTACA AAAAATATGT CCCAGAAAAA AAAAAAATCT TACCAAGATA 780

CGTAAAAAAA AAAAAAAAAA 800

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1810 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCAGCTCCCA GGTGCGTGTT AAAAGCTGGA GGGGGGATAT GTGATCCCAG GACCAAAAGC 60

GCGGGGCCAG ACTCATCGGT TCATTCAACA ACCAGTATTT AGTGCCTGCT GTGTTCTGCA 120

GGCCCTGCCA TAGGCGCTTG ATACAGCGGT GCATAGCGTA TGAAAAAGAT CTGTCCTGGC 180

TGAGCATCCG TAATATAAAA ATCTGAAATC TGAAATGCTC CAAAATCCTA AACTTTTTGA 240

GTGCTGACAT TATGCCACAA ATGGAAAATT TCATACCTGA CCTTATGTGG GTTGCANTCA 300

AAACACAGGT GCACAACACC CAGTTCATGC AACATCCCCA ATGGGAAAAA AGACCCCCCC 360

AGCTCTCTTC TGCTGCAGTT TTTCTGCTCA CACCTGGATT TCCCCATGCA TTCCCACAAA 420

AAGTAATTAA ATGGCATGCG TGCAGGCTGG ACACGCCAAC AACAGGTTTC CCACAATGCC 480

CCACATGGGG CCAAGACCTG TGTGCATTAC TCATTGCATT TTTTTGCTTA TTCTCTGCTG 540

TGTGGTATAA ATATATTGTT GAAAATGTCA AAAAGACCTA AAGATACCCC TGTGAATATC 600

AGTGATAAGA AAAAGAGGAA GCATTTATGT TTATCTATAG CACAGAAAGT CAAGTTGTTG 660

GAGAAACTGG ACAGTGGTGT AAGTGTGAAA CATCTTACAG AAGAGTATGG TGTTGGAATG 720

ACCACCATAT ATGACCTGAA GAAACAGAAG GATAAACTGT TGAAGTTTTA TGCTGAAAGT 780

GATGAGCAGA TATTAATGAA AAATAGAAAA ACACTTCATA AAGCTAAAAA TGAAGATCTT 840

GATCGTGTAT TGAAAGAGTG GATCCGTCAG CGTCGCAGTG AACACATGCC ACTTAATGGT 900

ATGCTGATCA TGAAACAAGC AAAGATATAT CACAATGAAC TAAAAATTGA GGGGAACTGT 960

GAATATTCAA CAGGCTGGTT GCAGAAATTT AAGAAAAGAC ATGGCATTAA ATTTTTAAAG 1020

ACTTGTGGCA ATAAAGCATC TGCTGGTCAT GAAGCAACAG AGAAGTTTAC TGGCAATTTC 1080

AGTAATGATG ATGAACAAGA TGGTAACTTT GAAGGATTCA NTATGTCAAG TGAGAAAAAA 1140

ATAATGTCTG ACCTCCTTAC ATATACAAAA AATATACATC CAGAGACTGT CAGTAAGCTG 1200

GAAGAAGAGG ATATCTTTNA TGTTTTTAAC AGTAATAATG AGGCTCCAGT TGTTCATTCA 1260

TTGTCCAATG GTGAAGTAAC AAAAATGGTT CTGAATCAAG ATGATCATGA TGATAATGAT 1320

AATGAAGATG ATGTTAACAC TGCAGAAAAA GTGCCTATAG ACGACATGGT AAAAATGTGT 1380

GATGGGCTTA TTAAAGGACT AGAGCAGCAT GCATTCATAA CAGAGCAAGA AATCATGTCA 1440

GTTTATAAAA TCAAAGAGAG ACTTCTAAGA CAAAAAGCAT CATTAATGAG GCAGATGACT 1500

CTGAAAGAAA CATTTAAAAA AGCCATCCAG AGGAATGCTT CTTCCTCTCT ACAGGACCCA 1560

CTTCTTGGTC CCTCAACTGC TTCTGATGCT TCTTCTCACC TAAAAATAAA ATAAAATACA 1620

GTGTACAGTA ACCTTTTAGT CAAAACAGCA TCATACTTGG AAACTGAAAG CCTACTGTTA 1680

TTTGTTATTG TTGCTTAACA GCTGATACAG GTATTCTGGT GACACTACTG TGCTGGCTTA 1740

CTTAACCTGA ATACACTATT TTTTTCGTTG TAAAAAAAAA AAAAAAANAA NAAAAAAAAA 1800

AAAAAANANA 1810

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Ala Arg Glu Gly Gly Lys Met Val Leu Glu Ser Thr Met Val Cys Val
1               5                  10                  15

Asp Asn Ser Glu Tyr Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu
            20                  25                  30

Gln Ala Gln Gln Asp Ala Val Asn Ile Xaa Cys His Ser Lys Thr Arg
        35                  40                  45

Ser Asn Pro Glu Asn Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys
    50                  55                  60

Glu Val Leu Thr Thr Leu
65                  70
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Ala Arg Glu Ser Thr Met Val Cys Val Asp Asn Ser Glu Tyr Met Arg
1               5                  10                  15

Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln Gln Asp Ala Val
            20                  25                  30

Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro Glu Asn Asn Val
        35                  40                  45

Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu Thr Thr Leu Thr
    50                  55                  60

Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr Val Gln Pro Lys
65                  70                  75                  80

Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala His Leu Ala Leu
                85                  90                  95
```

Lys His Arg Gln
100

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGCACGAGA AGGTGGCAAG ATGGTGTTGG AAAGCACTAT GGTGTGTGTG GACAACAGTG 60

AGTATATGCG GAATGGAGAC TTCTTACCCA CCAGGCTGCA GGCCCAGCAG GATGCTGTCA 120

ACATANTTTG TCATTCAAAG ACCCGCAGCA ACCCTGAGAA CAACGTGGGC CTTATCACAC 180

TGGCTAATGA CTGTGAAGTG CTGACCACAC TCAC 214

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 375 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TATGGACACA TTTGAGCCAG CCAAGGAGGA GGATGATTAC GACGTGATGC AGGACCCCGA 60

GTTCCTTCAG AGTGTCCTAG AGAACCTCCC AGGTGTGGAT CCCAACAATG AAGCCATTCG 120

AAATGNTATG GGCTCCCTGG CCTCCCAGGC CACCAAGGAC GGCAAGAAGG ACAAGAAGGA 180

GGAAGACAAG AAGTGAGACT GGAGGGAAAG GGTAGCTGAG TCTGCTTAGG GGACTGCATG 240

GGAAGCACGG AATATAGGGT TAGATGTGTG TTATCTGTAA CCATTACAGC CTAAATAAAG 300

CTTGGCAACT TTTTAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 360

AAAAAAAAAC TCGAG 375

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 304 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGCACGAGA AAGCACTATG GTGTGTGTGG ACAACAGTGA GTATATGCGG AATGGAGACT 60

TCTTACCCAC CAGGCTGCAG GCCCAGCAGG ATGCTGTCAA CATAGTTTGT CATTCAAAGA 120

CCCGCAGCAA CCCTGAGAAC AACGTGGGCC TTATCACACT GGCTAATGAC TGTGAAGTGC 180

TGACCACACT CACCCCAGAC ACTGGCCGTA TCCTGTCCAA GCTACATACT GTCCAACCCA 240

AGGGCAAGAT CACCTTCTGC ACGGGCATCC GCGTTGCCCA TCTGGCTCTG AAGCACCGAC 300

AAGG 304

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Val Arg Gly Gly Gly Gly Gly Gly Pro Gly Gly Gly Gly Val Gly Gly
1 5 10 15

Arg Cys Gly Gly Gly Gly
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Arg Ala Ala Arg Ala Lys Ala Gln Ala Leu Ile Gln Asn Leu Ser
1 5 10 15

Leu Leu Leu Val Asp Ala Ser Val Gly Thr Ile Gln Cys Leu Glu Glu
20 25 30

Ile Leu Cys Glu Phe Val Gln Lys Asp Glu Leu Lys Pro Ala Val Thr
35 40 45

Xaa Leu Leu Trp Glu Arg Ala Thr Glu Lys Val Ala Cys Cys Pro Leu
50 55 60

Glu Arg Cys Ser Ser Val Met Leu Leu Gly Met Met Ala Arg
65 70 75

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Lys Met Val Leu Glu Ser Thr Met Val Cys Val Asp Asn Ser Glu Tyr
1 5 10 15

Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln Gln Asp
20 25 30

Ala Val Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro Glu Asn
35 40 45

Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu Thr Thr
50 55 60

Leu Thr Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr Val Gln
65 70 75 80

Pro Lys Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala His Leu
85 90 95

Ala Leu Lys His Arg Gln Gly Lys Asn His Lys Met Arg Ile Ile Ala
100 105 110

Phe Val Gly Ser Pro Val Glu Asp Asn Glu Lys Asp Leu Val Lys Leu
115 120 125

Ala Lys Arg Leu Lys Lys Glu Lys Val Asn Val Asp Ile Ile Asn Phe
130 135 140

Gly Glu Glu Glu Val Asn Thr Glu Lys Leu Thr Ala Phe Val Asn Thr
145 150 155 160

Leu Asn Gly Lys Asp Gly Thr Gly Ser His Leu Val Thr Val Pro Pro
165 170 175

Gly Pro Ser Leu Ala Asp Ala Leu Ile Ser Ser Pro Ile Leu Ala Gly
180 185 190

Glu Gly Gly Ala Met Leu Gly Leu Gly Ala Ser Asp Phe Glu Phe Gly
195 200 205

Val Asp Pro Ser Ala Asp Pro Glu Leu Ala Leu Ala Leu Arg Val Ser
210 215 220

Met Glu Glu Gln Arg Gln Arg Gln Glu Glu Glu Ala Arg Arg Ala Ala
225 230 235 240

Ala Ala Ser Ala Ala Glu Ala Gly Ile Ala Thr Thr Gly Thr Glu Asp
245 250 255

Ser Asp Asp Ala Leu Leu Lys Met Thr Ile Ser Gln Gln Glu Phe Gly
260 265 270

Arg Thr Gly Leu Pro Asp Leu Ser Ser Met Thr Glu Glu Glu Gln Ile
275 280 285

Ala Tyr Ala Met Gln Met Ser Leu Gln Gly Ala Glu Phe Gly Gln Ala
290 295 300

Glu Ser Ala Asp Ile Asp Ala Ser Ser Ala Met Asp Thr Ser Glu Pro
305 310 315 320

Ala Lys Glu Glu Asp Asp Tyr Asp Val Met Gln Asp Pro Glu Phe Leu
325 330 335

Gln Ser Val Leu Glu Asn Leu Pro Gly Val Asp Pro Asn Asn Glu Ala
340 345 350

Ile Arg Asn Ala Met Gly Ser Leu Pro Pro Arg Pro Pro Arg Thr Ala
355 360 365

Arg Arg Thr Arg Arg Arg Lys Thr Arg Ser Glu Thr Gly Gly Lys Gly
370 375 380

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Arg Asp Ala Tyr Ser Phe Ser Arg Lys Ile Thr Glu Ala Ile Gly
1 5 10 15

Ile Ile Ser Lys Met Met Tyr Glu Asn Thr Thr Thr Val Val Gln Glu
20 25 30

Val Ile Glu Phe Phe Val Met Val Phe Gln Phe Gly Val Pro Gln Ala
35 40 45

Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile Trp Ser Lys Glu Pro
50 55 60

Gly Val Arg Glu
65

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Arg Ala Gln Ala Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile
1 5 10 15

Trp Ser Lys Glu Pro Gly Val Arg Glu Ala Val Leu Asn Ala Tyr Arg
20 25 30

Gln Leu Tyr Leu Asn Pro Lys Gly Asp Ser Ala Arg Ala Lys Ala Gln
35 40 45

Ala Leu Ile Gln Asn Leu Ser Leu Leu Leu Val Asp Ala Ser Val Gly
50 55 60

Thr Ile Gln Cys Leu Glu Glu Ile Leu Cys Glu Phe Val Gln Lys Asp
65 70 75 80

Glu Leu Lys Pro Ala Val Thr Gln Leu Leu Trp Glu Pro Ala Thr Glu
85 90 95

Lys

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Arg Ala Thr Thr Ala Phe Gly Cys Arg Ile Trp Asn Pro Cys Ala
1 5 10 15

Ala Leu Thr Met Lys Gln Ser Ser Asn Val Pro Ala Phe Leu Ser Lys
20 25 30

Leu Trp Thr Leu Val Glu Glu Thr His Thr Asn Glu Phe Ile Thr Trp
35 40 45

Ser Gln Asn Gly Gln Ser Phe Leu Val Leu Asp Glu Gln Arg Phe Ala
50 55 60

Lys Glu Ile Leu Pro Lys Tyr Phe Lys His Asn Asn Met Ala Ser Phe
65 70 75 80

Val Arg Gln Leu Asn Met Tyr Gly Phe Arg Lys Val Ile His Ile Asp
85 90 95

Ser Gly Ile Val Lys Gln Glu Arg Asp Gly Pro Val Glu Phe Gln His
100 105 110

Pro Tyr Phe Gln
115

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Arg Gly Ala Thr Cys Glu Arg Cys Lys Gly Gly Phe Ala Pro Ala
1 5 10 15

Glu Lys Ile Val Asn Ser Asn Gly Glu Leu Tyr His Glu Gln Cys Phe
20 25 30

Val Cys Ala Gln Cys Phe Gln Gln Phe Pro Glu Gly Leu Phe Tyr Glu
35 40 45

Phe Glu Gly Arg Lys Tyr Cys Glu His Asp Phe Gln Met Leu Phe Ala
50 55 60

Pro Cys Cys His Gln Cys Gly Glu Phe Ile Ile Gly Arg Val Ile Lys
65 70 75 80

Ala Met Asn Asn Ser Trp His Pro Glu Cys Phe Arg Cys Asp Leu Cys
85 90 95

Gln Glu Val Leu Ala Asp Ile Gly Phe Val Lys Asn Ala Gly Arg His
100 105 110

Leu Cys Arg Pro Cys His Asn Arg Glu Lys Ala Arg
115 120

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 768 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TACGAGGAGG AGGAGGAGGA GGCCCCGGAG GAGGAGGCGT TGGAGGTCGA TGCGGAGGCG 60

GAGGATGAGG AGGCCGAGGC GCCGGAGGAG GCCGAGGCGC CGGAGCAGGA GGAGGCCGGC 120

CGGAGGCGGC ATGAGACGAG CGTGGCGGCC GCGGCTGCTC GGGGCCGCGC TGGTTGCCCA 180

TTGACAGCGG CGTCTGCAGC TCGCTTCAAG ATGGCCGCTT GGCTCGCATT CATTTTCTGC 240

TGAACGACTT TTAACTTTCA TTGTCTTTTC CGCCCGCTTC GATCGCCTCG CGCCGGCTGC 300

TCTTTCCGGG ATTTTTTATC AAGCAGAAAT GCATCGAACA ACGAGAATCA AGATCACTGA 360

GCTAAATCCC CACCTGATGT GTGTGCTTTG TGGAGGGTAC TTCATTGATG CCACAACCAT 420

AATAGAATGT CTACATTCCT TCTGTAAAAC GTGTATTGTT CGTTACCTGG AGACCAGCAA 480

GTATTGTCCT ATTTGTGATG TCCAAGTTCA CAAGACCAGA CCACTACTGA ATATAAGGTC 540

AGATAAAACT CTCCAAGATA TTGTATACAA ATTAGTTCCA GGGCTTTTCA AAAATGAAAT 600

GAAGAGAAGA AGGGATTTTT ATGCAGCTCA TCCTTCTGCT GATGCTGCCA ATGGCTCTAA 660

TGAAGATNGA GGAGAGGTTG CAGATGAAGA TAAGAGAATT ATAACTGATG ATGAGATAAT 720

AAGCTTATCC ATTGAATTCT TTGACCAGAA CAGATTGGAT CGGAAAGT 768

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTAAATAAA CCAGCAGGTT GCTAAAAGAA GGCATTTTAT CTAAAGTTAT TTTAATAGGT 60

GGTATAGCAG TAATTTTAAA TTTAAGAGTT GCTTTTACAG TTAACAATGG AATATGCCTT 120

CTCTGCTATG TCTGAAAATA GAAGNTATTT ATTATGAGCT TNTACAGGTA TTTTTAAATA 180

GAGCAAGCAT GTTGAATTTA AAATATGAAT AACCCCACCC AACAATTTTC AGTTTATTTT 240

TTGCTTTGGT CGAACTTGGT GTGTGTTCAT CACCCATCAG TTATTTGTGA GGGTGTTTAT 300

TCTATATGAA TATTGTTTCA TGTTTGTATG GGAAAATTGT AGCTAAACAT TTCATTGTCC 360

CCAGTCTGCA AAAGAAGCAC AATTCTATTG CTTTGTCTTG CTTATAGTCA TTAAATCATT 420

ACTTTTACAT ATATTGCTGT TACTTCTGCT TTCTTTAAAA ATATAGTAAA GGATGTTTTA 480

TGAAGTCACA AGATACATAT ATTTTTATTT TGACCTAAAT TTGTACAGTC CCATTGTAAG 540

TGTTGTTTCT AATTATAGAT GTAAAATGAA ATTTCATTTG TAATTGGAAA AAATCCAATA 600

AAAAGGATAT TCATTTAAAA AAAAAAAAAA AAAAAAAAAA AA 642

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 236 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGCACGAGC TGCCAGAGCC AAGGCCCAGG CTTTGATTCA GAATCTCTCT CTGCTGCTAG 60

TGGATGCCTC GGTTGGGACC ATTCAGTGTC TTGAGGAAAT TCTCTGTGAG TTTGTGCAGA 120

AGGATGAGTT GAAACCAGCA GTGACCCANC TGCTGTGGGA GCGGGCCACC GAGAAAGTCG 180

CCTGCTGTCC TCTGGAACGC TGTTCCTCTG TCATGCTTCT TGGCATGATG GCACGA 236

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCGGGCGTAT TGGCGTGCGC CTGTAATCCC AGCTAACTCA AGAGGCTGAG GCAGGAGAAT 60

CGCCTGAACC CAGAGGCGGA GGTTGTAGTG AGCCGAAATC ACACCATTGC ACTCCAGCTT 120

GGGCAACAAT AGCGAACCTC CATCTCAAAT TAAAAAAAAA AATGCCTACA CGCTCTTTAA 180

AATGCAAGGC TTTCTCTTAA ATTAGCCTAA CTGAACTGCG TTGAGCTGCT TCAACTTTGG 240

AATATATGTT TGCCAATCTC CTTGTTTTCT AATGAATAAA TGTTTTTATA TACTTTTAGA 300

AAAAAAAAAA AAAAAAAAAA AAAAAAACTC GAG 333

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1272 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCAAGATGGT GTTGGAAAGC ACTATGGTGT GTGTGGACAA CAGTGAGTAT ATGCGGAATG 60

GAGACTTCTT ACCCACCAGG CTGCAGGCCC AGCAGGATGC TGTCAACATA GTTTGTCATT 120

CAAAGACCCG CAGCAACCCT GAGAACAACG TGGGCCTTAT CACACTGGCT AATGACTGTG 180

AAGTGCTGAC CACACTCACC CCAGACACTG GCCGTATCCT GTCCAAGCTA CATACTGTCC 240

AACCCAAGGG CAAGATCACC TTCTGCACGG GCATCCGCGT GGCCCATCTG GCTCTGAAGC 300

ACCGACAAGG CAAGAATCAC AAGATGCGCA TCATTGCCTT TGTGGGAAGC CCAGTGGAGG 360

ACAATGAGAA GGATCTGGTG AAACTGGCTA AACGCCTCAA GAAGGAGAAA GTAAATGTTG 420

ACATTATCAA TTTTGGGGAA GAGGAGGTGA ACACAGAAAA GCTGACAGCC TTTGTAAACA 480

CGTTGAATGG CAAAGATGGA ACCGGTTCTC ATCTGGTGAC AGTGCCTCCT GGGCCCAGTT 540

TGGCTGATGC TCTCATCAGT TCTCCGATTT TGGCTGGTGA AGGTGGTGCC ATGCTGGGTC 600

TTGGTGCCAG TGACTTTGAA TTTGGAGTAG ATCCCAGTGC TGATCCTGAG CTGGCCTTGG 660

CCCTTCGTGT ATCTATGGAA GAGCAGCGGC AGCGGCAGGA GGAGGAGGCC CGGCGGGCAG 720

CTGCAGCTTC TGCTGCTGAG GCCGGGATTG CTACGACTGG GACTGAAGAC TCAGACGATG 780

CCCTGCTGAA GATGACCATC AGCCAGCAAG AGTTTGGCCG CACTGGGCTT CCTGACCTAA 840

GCAGTATGAC TGAGGAAGAG CAGATTGCTT ATGCCATGCA GATGTCCCTG CAGGGAGCAG 900

AGTTTGGCCA GGCGGAATCA GCAGACATTG ATGCCAGCTC AGCTATGGAC ACATCTGAGC 960

CAGCCAAGGA GGAGGATGAT TACGACGTGA TGCAGGACCC CGAGTTCCTT CAGAGTGTCC 1020

TAGAGAACCT CCCAGGTGTG GATCCCAACA ATGAAGCCAT TCGAAATGCT ATGGGCTCCC 1080

TGCCTCCCAG GCCACCAAGG ACGGCAAGAA GGACAAGAAG GAGGAAGACA AGAAGTGAGA 1140

CTGGAGGGAA AGGGTAGCTG AGTCTGCTTA GGGGACTGCA TGGGAAGCAC GGAATATAGG 1200

GTTAGATGTG TGTTATCTGT AACCATTACA GCCTAAATAA AGCTTGGCAA CTTTTAAAAA 1260

AAAAAAAAAA AA 1272

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CGGCACGAGA TGCCTACAGC TTCTCCCGGA AGATTACAGA GGCCATTGGC ATCATCAGCA 60

AGATGATGTA TGAAAACACA ACTACAGTGG TGCAGGAGGT GATTGAATTC TTTGTGATGG 120

TCTTCCAATT TGGGGTACCC CAGGCCCTGT TTGGGGTGCG CCGTATGCTG CCTCTCATCT 180

GGTCTAAGGA GCCTGGTGTC CGGGAA 206

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TACTAAAAAT AAAAAATTAG CCGGGCGTAT TGGCGTGCGC CTGTAATCCC AGCTACTCAA 60

GAGGCTGAGG CAGGAGAATC GCCTGAACCC AGAGGCGGAG GTTGTAGTGA GCCGAAATCA 120

CACCATTGCA CTCCAGCTTG GGCAACAATA GCGAACCTCC ATCTCAAATT AAAAAAAAAA 180

TGCCTACACG CTCTTTAAAA TGCAAGGCTT TCTCTTAAAT TAGCCTAACT GAACTGCGTT 240

GAGCTGCTTC AACTTTGGAA TATATGTTTG CCAATCTCCT TGTTTTCTAA TGAATAAATG 300

TTTTTATATA CTTTTAANGA GAGAAAAAAA ANAAACTCGA G 341

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGCACGAGC CCAGGCCCTG TTTGGGGTGC GCCGTATGCT GCCTCTCATC TGGTCTAAGG 60

AGCCTGGTGT CCGGGAAGCC GTGCTTAATG CCTACCGCCA ACTCTACCTC AACCCCAAAG 120

GGGACTCTGC CAGAGCCAAG GCCCAGGCTT TGATTCAGAA TCTCTCTCTG CTGCTAGTGG 180

ATGCCTCGGT TGGGACCATT CAGTGTCTTG AGGAAATTCT CTGTGAGTTT GTGCAGAAGG 240

ATGAGTTGAA ACCAGCAGTG ACCCAGCTGC TGTGGGAACC GGCCACCGAG AAA 293

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGGCACGAGC TACCACCGCG TTCGGGTGTA GAATTTGGAA TCCCTGCGCC GCGTTAACAA 60

TGAAGCAGAG TTCGAACGTG CCGGCTTTCC TCAGCAAGCT GTGGACGCTT GTGGAGGAAA 120

CCCACACTAA CGAGTTCATC ACCTGGAGCC AGAATGGCCA AAGTTTTCTG GTCTTGGATG 180

AGCAACGATT TGCAAAAGAA ATTCTTCCCA AATATTTCAA GCACAATAAT ATGGCAAGCT 240

TTGTGAGGCA ACTGAATATG TATGGTTTCC GTAAAGTAAT ACATATCGAC TCTGGAATTG 300

TTAAGCAAGA AAGAGATGGT CCTGTAGAAT TTCAGCATCC TTACTTCCAA 350

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCCTAAAGCT TTCTCTGCTC CAGTTATTTT TATTAAATAT TTTTCACTTG GCTTATTTTT 60

AAAACTGGGA ACATAAAGTG CCTGTATCTT GTAAAACTTC ATTTGTTTCT TTTGGTTCAG 120

AGAAGTTCAT TTATGTTCAA AGACGTTTAT TCATGTTCAA CAGGAAAGAC AAAGTGTACG 180

TGAATGCTCG CTGTCTGATA GGGTTCCAGC TCCATATATA TAGAAAGATC GGGGGTGGGA 240

TGGGATGGAG TGAGCCCCAT CCAGTTAGTT GGACTAGTTT TAAATAAAGG TTTTCCGGTT 300

TGTGTTTTTT TGAACCATAC TGTTTAGTAA AATAAATACA ATGAATGTTG NAAAAAAAAA 360

AAAAAAAAAA ACTCGAG 377

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CGGCACGAGG CGCCACTTGC GAGCGCTGCA AGGGCGGCTT TGCGCCCGCT GAGAAGATCG 60

TGAACAGTAA TGGGGAGCTG TACCATGAGC AGTGTTTCGT GTGCGCTCAG TGCTTCCAGC 120

AGTTCCCAGA AGGACTCTTC TATGAGTTTG AAGGAAGAAA GTACTGTGAA CATGACTTTC 180

AGATGCTCTT TGCCCCTTGC TGTCATCAGT GTGGTGAATT CATCATTGGC CGAGTTATCA 240

AAGCCATGAA TAACAGCTGG CATCCGGAGT GCTTCCGCTG TGACCTCTGC CAGGAAGTTC 300

TGGCAGATAT CGGGTTTGTC AAGAATGCTG GGAGACACCT GTGTCGCCCC TGTCATAATC 360

GTGAGAAAGC CAGA 374

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 492 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTTTGCATTT TACAGTAAGA ATCAAAGTCC CTTCAGTGTG CCTTTGTCAG CTAATATGTG 60

ACCAGCAATG ACAACCTTGG GAGTATTTAT TAAATATTAT GCTATGAATA TAGGCAACAC 120

AGAACAGGGT TTGCAGTATA GCGTCTTGAT GCTAAATTCT CATATACCTC TACACGAGAA 180

ATATGGAGGA GAAAAACAAG CATTTACATA TATTCTTCGT CACTTTGAAG ATGCATGACC 240

TGAACTCGAC TGCTTGTGTT TGTTTACATA TCAGGCATAC CCAGGCATCT CCTGCAGCCA 300

GAGGTTCCAT TGCTGTCTTT GCTCAGTCCT CTTTTAAAAT ATGAATTAGT GGACAGGCAC 360

GGTGCCTCAC ACCTGTAATC CCAGCACTTT GGGAGGTCGA GGCAGGTGGA TCACGAGGTC 420

AGGAGATCAA GACCATCCTG GCTACCACTG AAACCCCATC TCTACTACAA AAAAAAAAAA 480

AAAAAACTCG AG 492

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ser Gln Ile Cys Glu Leu Val Ala His Glu Thr Ile Ser Phe Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Xaa Xaa Xaa Xaa Xaa Ser Ile Leu Asp Glu Val Ile Arg Gly Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Val Val Lys Thr Tyr Leu Ile Ser Ser Ile Pro Gln Gly Ala Phe Asn
1 5 10 15

Tyr Lys Tyr Thr Ala
20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Val Val Lys Thr Tyr Leu Ile Ser Ser Ile Pro Leu Gln Ala Phe Asn
1 5 10 15

Tyr Lys Tyr Thr Ala
20

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Xaa Ala Lys Lys Phe Leu Asp Ala Glu His Lys Leu Asn Phe Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Xaa Xaa Xaa Lys Ile Lys Lys Phe Ile Gln Glu Asn Ile Phe Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Xaa Lys Val Lys Val Gly Val Asn Gly Phe Gly Arg Ile Gly Arg Leu
1 5 10 15

Val Thr

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Xaa Tyr Gln Tyr Pro Ala Leu Thr Xaa Glu Gln Lys Lys Glu Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Xaa Pro Ala Val Tyr Phe Lys Xaa Xaa Phe Leu Asp Xaa Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Xaa Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly Asp Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Xaa Xaa Val Ala Val Leu Xaa Ala Ser Xaa Xaa Ile Gly Gln Pro Leu
1 5 10 15

Ser Leu

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Val Val Lys Thr Tyr Leu Ile Ser Xaa Ile Pro Leu Gln Gly Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Xaa Xaa Lys Thr Tyr Leu Ile Ser Ser Ile Pro Leu Gln Gly Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Asp Ile Pro Gln Thr Lys Gln Asp Leu Glu Leu Pro Lys Leu
1 5 10 15

CLAIMS

1. A polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 2, 4, 5, 6, 7 and 8, or a variant of said protein that differs only in conservative substitutions and/or modifications.

2. A polypeptide comprising an immunogenic portion of a prostate protein or a variant of said protein that differs only in conservative substitutions and/or modifications wherein said protein comprises an amino acid sequence of a portion thereof encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos. 11 and 13-19, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos. 11 and 13-19, or a complement thereof under moderately stringent conditions.

3. A DNA molecule comprising a nucleotide sequence encoding the polypeptide of claims 1 or 2.

4. An expression vector comprising the DNA molecule of claim 3.

5. A host cell transformed with the expression vector of claim 4.

6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cell lines.

7. A pharmaceutical composition comprising the polypeptide of claims 1 or 2 and a physiologically acceptable carrier.

8. A vaccine comprising the polypeptide of claims 1 or 2 and a non-specific immune response enhancer.

9. The vaccine of claim 8 wherein the non-specific immune response enhancer is an adjuvant.

10. A vaccine comprising a DNA molecule and a non-specific immune response enhancer, the DNA molecule comprising a nucleotide sequence encoding the polypeptide of claims 1 or 2.

11. The vaccine of claim 10 wherein the non-specific immune response enhancer is an adjuvant.

12. A pharmaceutical composition for the treatment of prostate cancer comprising a polypeptide and a physiologically acceptable carrier, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57.

13. A vaccine for the treatment of prostate cancer comprising a polypeptide and a non-specific immune response enhancer, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57.

14. The vaccine of claim 13 wherein the non-specific immune response enhancer is an adjuvant.

15. A pharmaceutical composition according to claim 7, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

16. A vaccine according to claim 8, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

17. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample obtained from a patient with a binding agent which is capable of binding to the polypeptide of claims 1 or 2; and

(b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting prostate cancer in the patient.

18. The method of claim 17 wherein the binding agent is a monoclonal antibody.

19. The method of claim 17 wherein the binding agent is a polyclonal antibody.

20. A method for monitoring the progression of prostate cancer in a patient, comprising:

(a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to the polypeptide of claims 1 or 2;

(b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b); and

(d) comparing the amount of polypeptide detected in steps (b) and (c) to monitor the progression of prostate cancer in the patient.

21. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample obtained from a patient with a binding agent which is capable of binding to a polypeptide, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57; and

(b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting prostate cancer in the patient.

22. The method of claim 21 wherein the binding agent is a monoclonal antibody.

23. The method of claim 21 wherein the binding agent is a polyclonal antibody.

24. A method for monitoring the progression of prostate cancer in a patient, comprising:

(a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to a polypeptide, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of: SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57;

(b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b); and

(d) comparing the amount of polypeptide detected in steps (b) and (c) to monitor the progression of prostate cancer in the patient.

25. A monoclonal antibody that binds to the polypeptide of claims 1 or 2.

26. A monoclonal antibody according to claim 25, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

27. The monoclonal antibody of claim 26 wherein the monoclonal antibody is conjugated to a therapeutic agent.

28. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample from a patient with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the

oligonucleotide primers is specific for a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and

(b) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primer, thereby detecting prostate cancer.

29. The method of claim 28, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

30. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample from the patient with at least one oligonucleotide probe specific for a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and

(b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting prostate cancer.

31. The method of claim 30 wherein the probe comprises at least about 15 contiguous nucleotides of a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

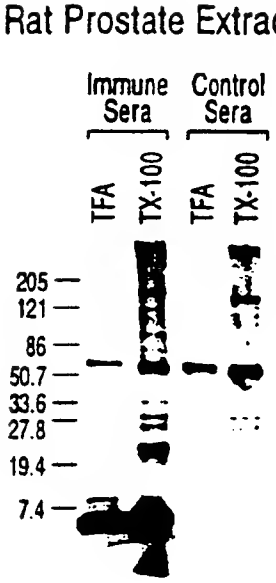
Rat Prostate Extracts
Immune Sera
Control Sera
TFA
TX-100
TFA
TX-100
205
121
86
50.7
33.6
27.8
19.4
7.4

Fig. 1

Rat Prostate Extract

Non-reduced SDS-PAGE

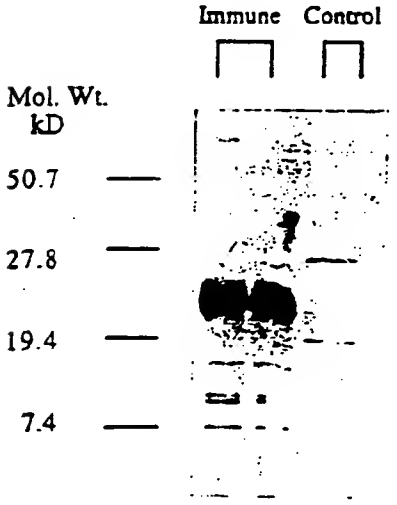

Fig. 2

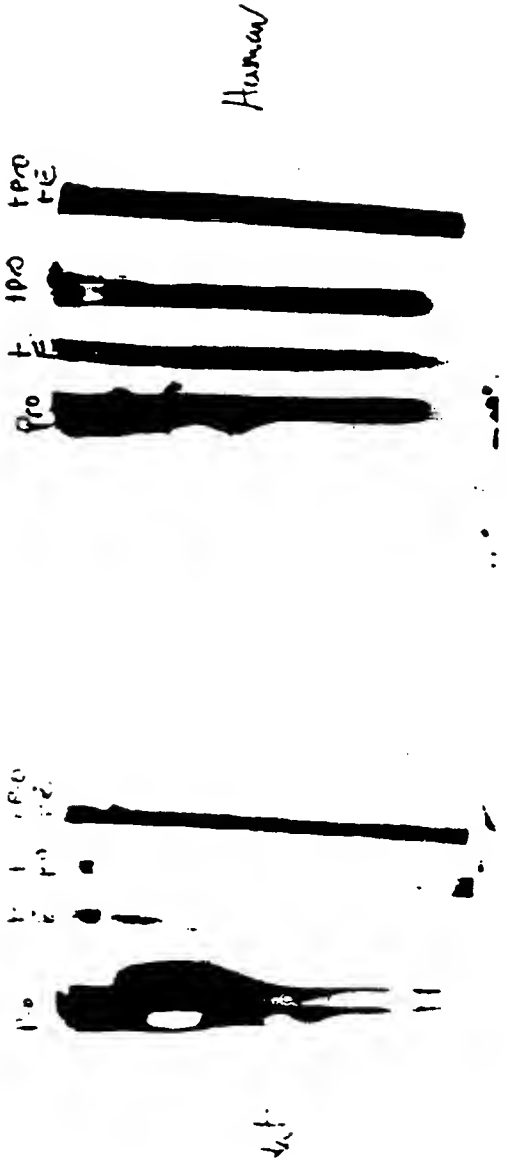

Fig. 3

CORRECTED VERSION*

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, A61K 38/17, 39/00, C12Q 1/68, G01N 33/68, C07K 16/18, 14/47, A61K 39/395 C12N 1/21

A3

(11) International Publication Number: **WO 97/33909**

(43) International Publication Date: 18 September 1997 (18.09.97)

(21) International Application Number: PCT/US97/04192

(22) International Filing Date: 14 March 1997 (14.03.97)

(30) Priority Data:

08/616,745	15 March 1996 (15.03.96)	US
08/633,840	11 April 1996 (11.04.96)	US

(71) Applicant: CORIXA CORPORATION [US/US]; Suite 464, 1124 Columbia Street, Seattle, WA 98104 (US).

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(74) Agents: MAKI, David, J. et al.; Seed and Berry L.L.P., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States: AL, AM, AT, AU, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 24 December 1997 (24.12.97)

(54) Title: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

(57) Abstract

Compounds and methods for treating and diagnosing prostate cancer are provided. The inventive compounds include polypeptides containing at least a portion of a prostate protein. Vaccines and pharmaceutical compositions for immunotherapy of prostate cancer comprising such polypeptides or DNA molecules encoding such polypeptides are also provided. The inventive polypeptides may also be used to generate antibodies useful for the diagnosis and monitoring of prostate cancer. Nucleic acid sequences for preparing probes, primers, and polypeptides are also provided.

* (Referred to in PCT Gazette No. 51/1997, Section II)

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INTERNATIONAL SEARCH REPORT

Inten ›nal Application No
PCT/US 97/04192

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 A61K38/17 A61K39/00 C12Q1/68 G01N33/68
C07K16/18 C07K14/47 A61K39/395 C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 09820 A (SLOANKETTERING INSTITUTE FOR CANCER RESEARCH) 11 May 1994 see the whole document especially see page 43 - page 44 see page 54 - page 60 see page 86 see page 51 - page 53 see sequences ID 1 and ID 2 ---	1-11, 15-20, 25-31
A	EL-SHIRBINY A M: "PROSTATIC SPECIFIC ANTIGEN" ADVANCES IN CLINICAL CHEMISTRY, vol. 31, 1994, pages 99-133, XP000617158 see the whole document --- -/--	1-11, 15-20, 25-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 1997	30. 10. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer LE CORNEC N.D.R.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/US 97/04192

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 04548 A (JENNER TECHNOLOGIES) 16 February 1995 see the whole document -----	1-11

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.rnational application No.
PCT/US 97/04192

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searcheble claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11; 15-20; 25-31 all partially (invention 1.)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the peyment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims (1-11) partially; (15-20) partially; 25-31 (partially)
   Prostate protein characterized by sequence ID2 and its nucleic acid sequence (ID11), expression vector, host cell, use of the protein in a pharmaceutical composition, antibody against the protein and its use in a method of diagnosis of prostate cancer and in a method of monitoring the progression of prostate cancer. Method of detection of prostate cancer using primers and probes derived from the nucleic acid sequence.
2. Claims (1-11) partially; (15-20) partially; (25-31) partially
   The same as defined above but for sequences ID4 and ID13, 14.
3. Claims (1-11) partially; (15-20) partially; (25-31) partially
   The same as defined above but for sequences ID5 and ID15
4. Claims (1-11) partially; (15-20) partially; (25-31) partially
   The same as defined above but for sequences ID6 and ID16
5. Claims (1-11) partially; (15-20) partially; (25-31) partially
   The same as defined above but for sequences ID7 and ID17,18
6. Claims (1-11) partially; (15-20) partially; (25-31) partially
   The same as defined above but for sequences ID8 and ID19
7. Claims (12-14) partially; (21-24) partially
   Pharmaceutical composition containing a prostate protein defined by sequence ID1. A method for detecting prostate cancer and a method for monitoring the progression of Prostate Cancer using an antibody against the prostate protein.
8. Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID3
9. Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID20
10 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID21
11 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID25
12 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID26
13 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID 27
14 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID 28
15 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID 29
16 Claims (12-14) partially; (21-24) partially
   The same as defined above but for sequence ID 30

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

17. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID31
18. Claims (12-14) partially: (21-24) partially
The same as defined above but for sequences ID44 and ID45
19. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID46 and ID47
20. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID48 and ID49
21. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID50
22. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID51
23. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID52
24. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID53
25. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID54
26. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID55 and ID56
27. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID57

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. nal Application No
PCT/US 97/04192

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9409820 A	11-05-94	CA 2147499 A	11-05-94
		EP 0668777 A	30-08-95
		JP 8506005 T	02-07-96
		US 5538866 A	23-07-96
WO 9504548 A	16-02-95	AU 7631294 A	28-02-95
		EP 0721345 A	17-07-96
		JP 9504000 T	22-04-97